The Effects of Powerboat Emissions on the Water Quality of Loch Lomond

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Important Note

Any opinions and conclusions expressed herein are entirely those of the author and are not necessarily representative of any of the funding bodies or collaborating agencies.

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Abstract

Recently, there has been increased concern about the potential impact of powerboating on freshwater ecosystems. In the case of Loch Lomond, such concern led to the commencement of a programme of boat counting surveys in 1989. In these surveys, it was found that maximum boat numbers are increasing in Loch Lomond. As a logical progression from these surveys, the present study examined the impact of powerboat chemical pollution on the water quality of Loch Lomond.

In the first stage of this study, the scale of powerboat pollution was assessed. This was achieved by constructing a simple mathematical model to estimate the total annual discharge of hydrocarbons (HCs) from powerboat emissions into Loch Lomond. By using boat census data and published information about the emission rates of different types of powerboat engines, the total annual input for Loch Lomond was estimated at 25.50 tonnes in 1989 and this input has greatly increased since then. This indicates that there is real potential for hydrocarbon (HC) pollution of Loch Lomond from powerboat operation. Most of the HC material discharged emanated from speedboats powered by outboard motors.

To identify and quantify HC pollutant compounds entering water from outboard engines, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed on water subject to a controlled pollution discharge from an outboard motor. Altogether, 47 compounds were detected in this exhaust-polluted water (EPW), with most of these being volatile aromatic HCs, such as benzene and alkylated benzenes.

Water samples were also taken from three sites in Loch Lomond on two occasions: once in winter, during negligible boat activity and once in summer, at a time of high powerboat activity. No HCs were detected in winter, but some volatile aromatic HC compounds were clearly detectable in summer. These compounds were the same as those most abundant in EPW and found in similar relative proportions.

Further experiments in this study involved the analysis of a large number of water samples. To enable this, it was necessary to develop an appropriate new method for the analysis of powerboat exhaust pollutants, which are mostly aromatic HC. All aromatic HCs fluoresce and the new method involved fluorescence spectroscopy analysis, following sample purification by normal-phase column chromatography. This technique is non-destructive allowing subsequent confirmatory analysis by GC-MS. It was found that the new method was fast, precise, highly sensitive and specific to volatile aromatic HCs.

The capability of measuring HCs in a large number of samples allowed previously unfeasible experiments investigating:

1) The geographical distribution of powerboat exhaust HCs in Loch Lomond.
2) The depth profile of powerboat exhaust pollutants in the top 1.5 m of the water column.
3) Recovery of water quality, following peak weekend boat activity in summer.

It was found that:

1) Volatile aromatic HCs from powerboat exhaust are detectable over much of Loch Lomond on days of heavy powerboat activity, with concentrations of up to 37 $\mu g.l^{-1}$ occurring.
2) Pollutant HCs are found at depths of at least 1.5 m, and are not confined to the surface microlayer (100 μm). The HC distribution with depth was mostly uniform in the field and this was confirmed in controlled experiments in a tank.
3) Recovery of water quality was difficult to demonstrate in the field, possibly as pollution incidents occur continually during summer.
4) HC concentration declined in controlled experiments, in which water was subjected by a controlled pollution discharge from an outboard motor. The time taken for the HC concentration to reach 50 % of the initial HC loading varied but was approximately 7 - 9 days.

The toxicity of EPW was investigated by performing 24 hour LC50 (Lethal Concentration for 50 % of test organisms) tests, using the water flea, *Daphnia magna* (an international standard test organism). The mean LC50 recorded in this study, expressed as the total concentrations of HC compounds, was 3.72 $mg.l^{-1}$.

Previous studies suggest that multiplying the LC50 for *D. magna* by an application factor of 0.001 gives an approximate safe level of a pollutant. Using data collected in this study, a safe level of exhaust HC compounds of 3.72 $\mu g.l^{-1}$ would result. In summer, during times of high powerboat activity, such levels are exceeded at many locations in Loch Lomond.

The current study has shown that HC pollution from a relatively small number of inefficient powered recreational craft using Loch Lomond poses a potential threat to the maintenance of water quality.

Chapter 1

Introduction

1.1: General Introduction

Historically, inland waterways have had important transport and communications functions. These uses have declined, but this decline has been accompanied by a dramatic increase in water based recreational activities, including powerboating (Murphy *et al.*, 1982; IWAAC, 1983). The probable causes of this change in use are increases in available leisure time and disposable income coupled with vastly improved transport links to recreational areas. This has led to renewed concern about the potential impact of powerboats on freshwater ecosystems (Murphy *et al.*, 1993; Mele, 1993).

The increase in powerboat use has been dramatic over the last forty years or so. Table 1.1 shows the increase in the numbers of powerboats propelled by inboard and two-stroke outboard motors and where known, the average horsepower of boats in the United States, the country for which the greatest amount of information is available on a nationwide scale. There has been a steady increase in the numbers of outboard motors accompanied by a sharp increase in mean engine size.

Faster boats with larger engines can cause more ecological damage in a number of ways:

- They produce a greater amount of wave energy, which can cause shore erosion.
- They can re-suspend bottom sediment more easily.
- They produce a greater amount of exhaust pollution.
- They are faster and noisier and thus have a greater potential to disturb wildlife.

Consequently, the environmental impact of boat activity is likely to have increased dramatically.

Table 1.1: Increases in powerboating activity in the United States, showing the numbers (in millions) of operational boats in the whole country, powered by two-stroke and four stroke motors and the mean power output of two-stroke motors (in horsepower).

Study	Year of census	Number of two-stroke outboard motors	Mean power output of two-stroke outboards	Number of four-stroke engines
Lagler *et al.* (1950)	1950	3.0		
English *et al.* (1963*a*)	1959	5.8	24	
Muratori (1968)	1966	6.8		0.7
Montz *et al.* (1982)	1979	7.0	44	
Mele (1993)	1989	8.0	68	4.0

Less is known about changes in powerboating in Europe in general and the UK in particular. Payne and Hey (1982) stated that the numbers of registered boats in the Norfolk Broads had more than trebled from 1947 to 1980. This includes sailing craft, and since their proportion of the total has declined over this period, the number of registered powered craft has increased by an even greater factor. Another study was carried out by the Lake District National Park Authority (LDNPA, 1994). The results of a one-off survey in 1977 were compared with those of later studies (albeit with different census methods). This study suggests that from 1977-1993, the numbers of powerboats using Lake Windermere approximately doubled. In other European countries, increases in recreational boating have also taken place. In Lake Constance, Switzerland, concern about the impact of such increases on the environment led to the imposition of a ban on the use of all engines with a power output of over 10 horsepower (Bartlett, 1989).

1.2: Environmental Effects of Powerboat Activity

Powerboats may have a number of effects on the freshwater environment. Table 1.2 lists these effects with examples of relevant studies.

Table 1.2: The effects of powerboats on aquatic ecosystems, with examples of previous relevant studies.

FACTORS	EFFECTS	EXAMPLES OF RELEVANT STUDIES
(1) Boat Wakes	Shore Erosion	Liddle and Scorgie (1980); Payne and Hey (1982); Murphy and Eaton (1983); Nanson *et al.* (1994)
	Wildfowl Disturbance	Tuite *et al.* (1983); Varney and Crooks (1989)
(2) Propeller and Jet Action	Direct Plant Damage	Lagler *et al.*, 1950; Murphy and Eaton (1983)
	Increase in Turbidity	Murphy and Eaton (1983); Garrad and Hey (1987)
	Direct Floral and Faunal Damage	Sutherland and Ogle (1975); Liddle and Scorgie (1980)
(3) Noise / Sight of Boats	Wildfowl disturbance	Hume (1976); Tuite *et al.* (1983)
	Decrease in Amenity Value	Ashton and Chubb (1972); Walker (1994)
(4) Launchings	Shore Vegetation Erosion	Rees and Tivy (1978)
(5) Fuel and Lubricant Oil Emissions and Spillage	Chemical Effects	Montz *et al.* (1982); US EPA (1991); Jüttner (1994)
	Ecotoxicological Effects	Kuzminski *et al.*(1972); Brenniman *et al.* (1979); Jüttner *et al.* (1995b)
(6) Boat Sewage Emissions (including illegal discharge of chemical toilets)	Organic Enrichment Pollution	Chmura and Ross (1978); Windermere Steering Committee (1981); Loch Lomond and the Trossachs Working Party (1993)
(7) Use of Surfactants (soaps and detergents)	Pollution (including synergism with oils)	Tetlow (undated); Lewis (1992); Mukherjee and Pankajakshi (1995)
(8) Use of Anti-Fouling Paints	Ecotoxicological Effects	Dowson *et al.* (1993, 1994);Dahl and Blanck (1996)
(9) Overcrowding	Perceived Decreases in Amenity Value by Recreational Users	Ashton and Chubb (1972); Walker (1994)
(10) Litter	Unsightliness; pollution	Dumbarton District Council *et al.* (1996); Tunstall *et al.* (1997)
General Reviews		Jackivicz and Kuzminski (1973); Liddle and Scorgie (1980)

1.2.1: Bank Erosion (by Boat-Induced Waves and Launching Activity)

This is the one of the most obvious environmental effects of powerboat activity. A number of studies have found critical wave heights (Payne and Hey, 1982; Nanson *et al.*, 1994), above which many types of river or lake margin become eroded. Management of waterways benefits from such knowledge as maximum speed limits can accordingly be set. Other studies (Murphy *et al.*, 1982; Murphy and Eaton, 1983) have found that in certain waterways, a low level of boat traffic can be beneficial in preventing nuisance growth of plants, while still not having any adverse effect on marginal vegetation. Heavier boat traffic, however, causes bank erosion and vegetation loss.

Loss or alteration of marginal vegetation can also occur as a result of boat launching activity. Rees and Tivy (1978) demonstrated that shoreline vegetation community structure differs in areas subject to different levels of boat launching activity, with vegetation being trampled and often lost in severely impacted areas.

1.2.2: Increases in Water Turbidity from Boats

In shallow water, the disturbance of bottom sediments and associated increase in water turbidity is an obvious effect of boat activity, and consequently most relevant research in this subject focuses on canals, rivers and shallow lakes. Increases in turbidity can have a number of far-reaching effects, including the prevention of growth of submerged macrophytes by depriving them of light (Murphy and Eaton, 1983). This can further weaken the sediment at the shoreline and make it susceptible to even more erosion, either by wind or waves, leading to further increases in turbidity. Garrad and Hey (1987) showed conclusively by means of controlled experiments, that boat activity increases turbidity. With heavy traffic, turbidity remains elevated throughout the day and only recovers at night, with cessation of boat activity.

1.2.3: Direct Floral and Faunal Damage

Propeller action can be responsible for direct damage of aquatic macrophytes by cutting stems and leaves (Liddle and Scorgie, 1980). Indeed, in shallow water, powered boat passage can remove plants completely (Lagler *et al.*, 1950). Another related consequence of the activity of boats is their capacity to assist in the dispersal of seeds or vegetative fragments of plants. This can be undesirable in situations involving introductions of alien species (Johnstone *et al.*, 1985).

Since most animals are capable of effective escape responses, direct faunal damage by boats has not been frequently observed. The most well documented case is the Florida manatee (*Trichechus manatus latirostris*), which is a ponderous slow-moving aquatic mammal, not best able to escape fast powerboats. Previous studies have shown that the primary cause of mortality in Florida manatees is boat collisions (O'Shea *et al.*, 1985; Shackley, 1992; Marmontel *et al.*, 1997) and this has led to proposed protective legislation for the future protection of the species (Marmontel *et al.*, 1997). Few other species of animals are subject to direct physical damage by boats. In a rare relevant study (Sutherland and Ogle, 1975), it was found that jet-boats were capable of causing mortality in developing salmon eggs.

1.2.4: Wildlife Disturbance

Although, unlike plants, most animals are able to avoid the possibility of physical damage from boats, disturbance of wildlife by sight and noise of boats is clearly an undesirable effect and the degree to which this occurs can be a problem in some areas. Boat activity can have marked adverse consequences on wildfowl populations and this has been demonstrated in the case of Llangorse lake, Wales (Tuite *et al.*, 1983). Some species are more susceptible than others, and Hume (1976) demonstrated that in the UK, goldeneye were the most sensitive, taking to flight when boats approached within 350

metres. Problems have also been noted elsewhere; for example boat activity on the Gulkana River in Alaska was found to disturb breeding bald eagles, *Haliaeetus leucocephalus* (Steidl and Anthony, 1996). Very little work has been carried out on the disturbance of other species (including fish and mammals) by boats. In the only hitherto known study, Adams *et al.* (1997) were able to show that caged juvenile trout showed a pronounced escape response to passing boats, whether engine-powered or not, but only when they were within visual range of the fish.

1.2.5: Decreases in Aesthetic Qualities (from overcrowding, noise and litter)

High levels of recreational boat activity can also have a detrimental effect on people's enjoyment of waterways. In one study (Ashton and Chubb, 1972), an arbitrary measure of a lake's carrying capacity for recreation was calculated, based on the relationship between boat activity and user satisfaction. They found that a majority of respondents expressed dissatisfaction when boat densities exceed one boat per three hectares. In a more recent study, Walker (1994) classified recreational participants according to their different activities and then gauged satisfaction levels and perception of conflict between people pursuing various activities. It was found that the participants in different activities have markedly different opinions on whether other activities caused interference with their own and also which of these activities were most responsible.

Aesthetic issues are very subjective, particularly the factor of human perception of noise pollution. Previous studies (e.g. Birmingham City Council, 1989) have consistently shown that boats do not violate any legal noise limits, but by contrast even low levels of noise in a quiet setting can be perceived as undesirable. The most productive approach to any study on noise pollution could involve questionnaires to recreational users, particularly in known quiet areas.

As perceived by the general public, the most obvious form of pollution is that from litter (Tunstall *et al.*, 1997). Boats could contribute towards this, particularly in areas of boat launching and on waterways themselves. The best solution to this problem could involve encouraging boat users to take litter home with them, as recommended in recent plans for the management of the Norfolk Broads (Broads Authority, 1997) and Loch Lomond (Dumbarton District Council *et al.*, 1996). Such a solution would work best when allied to effective policing of the waterways.

1.2.6: Sewage Pollution from Boat Toilet Discharges

Over the past thirty years or so, there have been great improvements in the treatment of sewage discharged from land to freshwaters. In the UK, over 80% of sewage discharged currently undergoes at least secondary treatment. In addition, tertiary treatment, involving phosphate stripping, has been introduced to many sewage treatment works (Haslam, 1990). In the case of sewage discharged from boats, however, there has been a lack of restrictions. Concern has prompted recommendations for improving the facilities for boat toilet pump-out stations in some areas (Windermere Steering Committee, 1981; Loch Lomond and the Trossachs Working Party, 1993), but until matters improve, sewage pollution from boats may remain a threat to water quality and human health. This not only includes sewage discharges from boats exempt from the requirement to install boat chemical toilets, but also the illegal discharge of boat chemical toilets themselves, of which there is anecdotal evidence.

1.2.7: Surfactants (Soaps and Detergents)

Surfactants (or surface-active compounds) include ordinary soap and petroleum-derived detergents. The most common surfactant compound in use is in the latter category: Linear Alkylbenzene Sulphonate (LAS). In a review, Lewis (1992) found that

some petroleum-derived detergents, including LAS, can be highly toxic to aquatic organisms. In addition to this, they pose a threat by acting synergistically with oils, i.e. increasing their toxicity.

Small quantities of surfactants are inevitably released into freshwaters from boat sink discharges and as a consequence of boat washing activities (Tetlow, undated). If this input is significant, it could be more hazardous to aquatic life than expected from the surfactant concentrations alone, in the light of synergism between surfactants and oil. Adverse effects of land-derived surfactants on the growth of freshwater phytoplankton in the field have already been documented (Mukherjee and Pankajakshi, 1995)

1.2.8: Toxic Pollution by Antifouling Paints

The adverse effects of tri-butyl tin (TBT), a component of anti-fouling paint have been well studied (Clark, 1989; Wishovsky *et al.*, 1989; Rice and Weeks, 1990). The use of TBT in anti-fouling paints was banned in Britain in 1987 and in many other countries throughout the 1980's and since these laws have been implemented, there have been signs of recovery from this form of pollution both in freshwater (Dowson *et al.*, 1994; Fent and Hunn, 1995) and marine (Dowson *et al.*, 1993; Ritsema, 1994) ecosystems. New anti-fouling paints have since been developed, based on copper and s-triazine herbicides and significant copper contamination has already been attributed to these new paints in some areas (Wright and Zamuda, 1991). In another recent study, it was found that these new paints can be very toxic by release of the herbicides present in them, which even at very low concentrations (0.063-0.25 μg/l) can kill marine epiphytic algae (Dahl and Blanck, 1996). In that study, it was also thought that such concentrations were likely to occur in areas of heavy recreational impact.

1.3: Hydrocarbon Pollution from Powerboat Exhaust

1.3.1: Influence of Engine Structures and Types on Hydrocarbon Emissions from Powerboats

As with all internal combustion engines, including automobiles, powerboat engines emit exhaust which includes hydrocarbons from unburned fuel (Jackivicz and Kuzminski, 1973; Mele, 1993). Most powerboats are propelled by two-stroke outboard motors (Liddle and Scorgie, 1980; Mele, 1993; UK CEED, 1993) and there are some fundamental differences between the structure of these engines and other types of internal combustion engine, which are usually four-stroke engines. The pistons of two-stroke outboards have combined intake and exhaust strokes whereby large quantities of unburned fuel pass straight through the combustion chamber and out through ports which lead to the exhaust system. These ports are simply holes unlike the more efficient valves which are present in four-stroke engines (Mele, 1993). Intake and exhaust strokes are separate in four-stroke engines, allowing the fuel to burn much more thoroughly and efficiently. In addition, outboard motors also lack catalytic converters (which are now mandatory in the construction of all automobiles in Europe and North America) and fuel injection systems, both of which reduce hydrocarbon emissions drastically (French, 1990). As a result of all of these factors, a typical modern two-stroke outboard motor uses over ten times as much fuel and emits one hundred and forty times as much unburned hydrocarbon material as a modern automobile engine with the same power output (Mele, 1993).

Two-stroke outboard motors propel the great majority of powerboats in North America (Mele, 1993) and also a high proportion of those in the UK (Liddle and Scorgie, 1980; UK CEED, 1993). The remainder of powerboats are propelled by inboard engines (either petrol or diesel powered) and four-stroke outboards which have similar designs to automobile engines and are more efficient than two-stroke outboards. However, even inboard and four-stroke outboard motors still produce nearly ten times as much

hydrocarbon (HC) pollution as modern car engines (they also lack the aforementioned pollution reduction devices present in car engines) and their contribution to powerboat exhaust pollution of aquatic ecosystems may be significant.

1.3.2: Comparison of Powerboat Exhaust Hydrocarbon (HC) Pollution with Other Types of HC Pollution

There are numerous sources of HC pollution (Connell and Miller, 1984), with two of the most obvious being:

1) Pollution due to the extraction, transport and refinement of crude oil.
2) Pollution from automobiles.

The former type of pollution usually takes the form of oil spills, particularly of crude oil. There have been many studies on oil pollution (see NRC, 1985 for an overview) and there is great public awareness of this type of pollution, particularly marine oil spills. This is due to the obvious nature of the adverse effects of such pollution, particularly the oiling of seabirds. Examples of particular crude oil spills (with the amount of oil spilled in metric tonnes [t]) include:

- The *Amoco Cadiz*, 1978 (221,000 t – see Page *et al.*, 1988)
- The Gulf War, 1991 (≥720,000 t – see Sauer *et al.*, 1993)
- The *Braer*, 1993 (85,000 t – see Turrell, 1994).

Spillage of refined oils such as diesel oil have also taken place (e.g. Pople *et al.*, 1990; Clark *et al.*, 1990). Although smaller volumes of oil are typically involved in each incident and the effects slightly less obvious, the toxicity of refined oils (weight for weight) is considerably greater than that of crude oil (Gaur and Singh, 1989).

There is also great public awareness of air pollution from automobile exhaust (French, 1990). Urban air pollution from cars can impact adversely on human health and awareness of this has led to legislative pressure, which has largely succeeded in compelling car manufacturers to produce cleaner engines. This has been achieved both by making cars more fuel efficient and also by fitting pollution reduction devices, such as catalytic converters. These measures have reduced HC emissions from modern car engines to as little as one tenth of that of that of older (pre 1980) engines (Mele, 1993).

There is a lack of awareness about pollution from powerboat engine exhaust, possibly due to the less obvious nature of its effects than those of crude oil spills or vehicle exhaust. Despite this, there is evidence that the total amount of pollutants discharged annually by powerboats could represent a threat to the environment of similar magnitude to that from either of the aforementioned sources of HC pollution (Mele, 1993).

1.3.3: Previous Research in Powerboat Exhaust Pollution

The subject of pollution from powerboat exhaust has been somewhat neglected in Europe, with most studies being carried out in North America. The first major study of powerboat pollution and its effects was performed by Lagler *et al.* (1950). He subjected a lake to intensive powerboat operation and finding no visible signs of oil pollution, concluded that powerboats were not significant polluters. Most later studies tended to concur with this viewpoint, for example a large scale study by the United States Environmental Protection Agency (EPA) commissioned by the Boating Industry Associations (1975). That study concluded that, although water, polluted by outboard motor exhaust, was toxic to fish in high enough concentrations, these concentrations would never occur in the field. In addition, a large scale examination of pollution in artificial lakes failed to show any major chemical or biological effects. To this day many powerboating interests point to that study as proof that their activities are harmless to the

environment (Butcher, 1982; UK CEED, 1993). Indeed in one study (Greentech Research, undated), it was claimed that powerboat activity was beneficial to the environment!

Recently, however, these precepts have been challenged. Firstly, amid some controversy, accepted safe levels of aquatic pollutants have been revised downwards as a result of greater understanding of toxic effects. In a study by English *et al.* (1963*a*), safe levels of powerboat exhaust pollutants in the field were estimated by multiplying the lethal concentrations (in a fish toxicity test) by a completely arbitrary application factor of 0.1. In the Boating Industry Associations (1975) study, lethal concentrations of outboard motor exhaust pollutants, for goldfish, were nine times the highest level found in artificial lakes. Authors of this report interpret this as proof that harmful levels of powerboat pollutants did not occur in their test lakes, but stress that boating levels in the artificial lakes were estimated as three times the maximum occurring in a real world situation.

There have not been any more recent recommendations for safe levels of powerboat exhaust pollutant levels in aquatic ecosystems. Previous research (Jüttner, 1994, 1995*a*) found that powerboat exhaust consists of a complex mixture of HC pollutants. Recently, there have been few recommendations for safe levels of any complex mixture in the aquatic environment, although there have been recommendations for safe environmental levels of single compounds. Kooijman (1981), stated that safe levels of particular aquatic pollutant compounds were usually calculated by multiplying lethal concentrations by a factor of 0.01. More recently, the Organisation for Economic Co-operation and Development (OECD, 1992) has derived "environmental concern levels" by applying a factor of 0.001 to known lethal levels of particular pollutants, derived from a single lethal toxicity test. This is also the application factor recommended by the Council of the European Community (CEC, 1994*a*).

Over the past three decades, there have been steady improvements in the methods and equipment used for the detection and characterisation of organic chemicals emanating from powerboats. In an early study, English *et al.* (1963*a*) identified outboard motor pollutants as volatile and non-volatile oils, phenols and lead. Later the Boating Industry Associations (1975) identified various groups of aromatic HC compounds. A more recent study by Montz *et al.* (1982) used Gas Chromatography with a Flame Ionisation Detector (GC-FID) to identify the water soluble chemical components of outboard motor exhaust. This enabled the identification and quantification of eight aromatic compounds. Even more precise techniques are available, such as Gas Chromatography-Mass Spectrometry (GC-MS) which allows accurate identification and precise quantification of organic compounds, even in low concentrations. GC-MS was used in a very recent study by Jüttner (1994) in which 46 compounds were identified in water, polluted by a four-stroke outboard motor. The mixture of pollutants emanating from two-strokes was found to be almost identical with respect to the compounds present and their relative proportions, although two-stroke motors produce a far greater amount of HC pollution (Jüttner *et al.*, 1995*a*). GC-MS has also been successfully used in other studies of HC pollution, including the detailed analysis of automobile exhaust (Olson *et al.*, 1992) and crude oil samples (Page *et al.*, 1988).

Despite such advances in technology, some workers may not have had access to the best analytical techniques. In one study (Bartlett, 1989) in which aliphatic HCs in sediment were analysed, it was concluded that powerboat engine operation had no effect on overall HC concentrations in the sediment of the Bodensee (Lake Constance). Later research (Mastran *et al.*, 1994) demonstrated that powerboats can increase the concentrations of other types of HCs (including the highly toxic Polycyclic Aromatic Hydrocarbons or PAHs) in sediment, suggesting that Bartlett (1989) may not have analysed the appropriate indicators (in this case PAHs) of powerboat exhaust pollution in sediment.

1.4: Loch Lomond

1.4.1: General Description (of the Study Area)

With an area of 70.27 km^2, Loch Lomond is the largest freshwater lake in mainland Britain. It is located 30 km north west of Glasgow, between Latitude 56°00' and 56°19' N and Longitude 4°30' and 4°43' W. With a temperate maritime climate, typical of western Scotland, the loch rarely freezes over and its water column usually has just one period of vertical mixing per year, in autumn. The loch is thus classed as a warm monomictic lake (Habib *et al.*, 1997). Fig 1.1 graphically depicts the average annual pattern of wind direction for a nearby site for which data is available (for Glasgow Airport, less than 30 km away; data from the Met. Office, 1998). As can be seen, the prevailing wind is most often from a south-westerly direction.

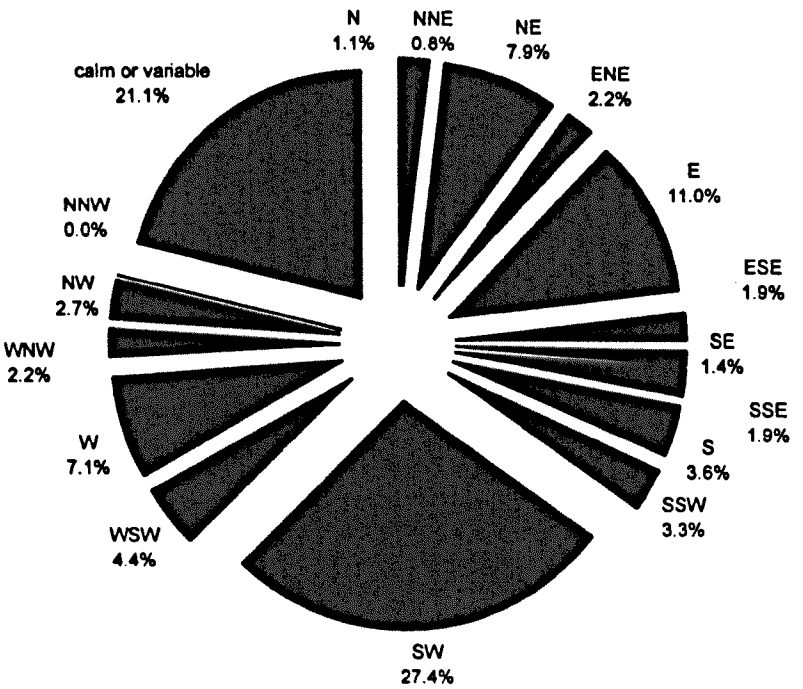

Fig. 1.1: Breakdown of prevailing wind direction throughout one calendar year (February 1997 to January 1998) for Glasgow Airport.

Despite being low-lying, with a mean surface water altitude of just 7.5 m above sea level, most of the loch's catchment is mountainous (see Fig. 1.2). However, uniquely for large Scottish lochs, it is crossed by the Highland Boundary Fault (H.B.F.) giving the loch a dual character. In the south, the loch is broad and shallow and the catchment for this section of the loch is mostly lowland agricultural. Table 1.3 presents a break-down of the major sources of inflowing water into Loch Lomond (data from Tippett, 1994). In the north, the loch has a typical appearance of a highland loch, being narrow and very deep in places (reaching depths of up to 190 metres). Fig. 1.2 shows a bathymetric map of the loch (from Tippett, 1994) with spot heights on land and inflowing rivers from the loch's catchment also indicated. There are even significant differences in weather conditions between the north and south extremities of the loch with annual rainfall in the north being considerably greater than that of the south (Tippett, 1994).

Table 1.3: A breakdown of the major sources of inflowing water into Loch Lomond (data from SEPA, West Region).

Origin	Mean Inflow (m3 x 106 y-1)	Percentage
Rivers north of H.B.F.	654	50.6
Small streams north of H.B.F.	214	16.6
Rivers south of H.B.F.	278	21.5
Rainfall on loch surface	146	11.3
Total	1292	100.0

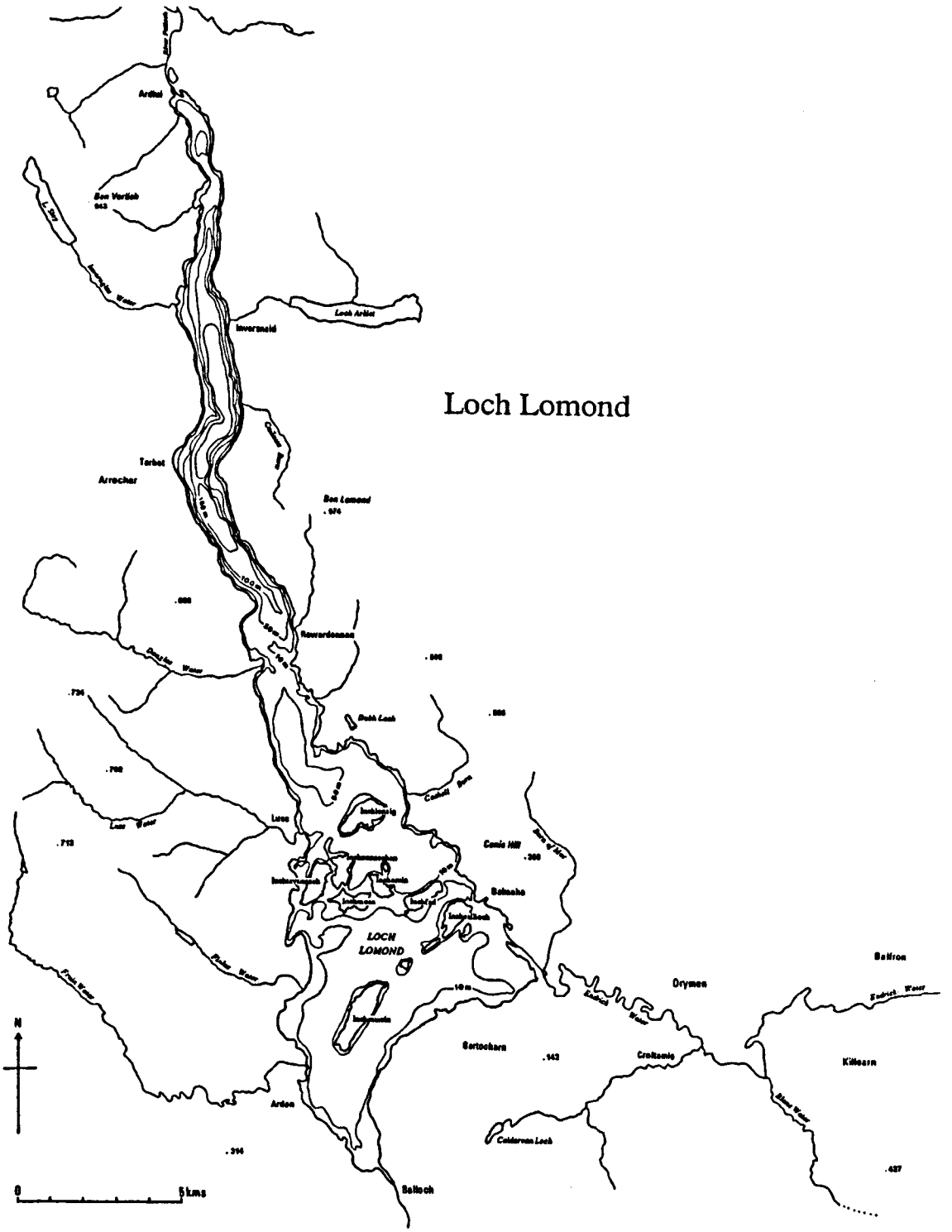

Fig. 1.2: Map of Loch Lomond and its catchment. Depth contours in the loch (in metres) are indicated, in addition to spot heights on land (in metres).

Although in overall terms, Loch Lomond is classed as an oligotrophic lake, the dual character of the loch's catchment is also reflected in its water chemistry, the waters of the South Basin being considerably more nutrient rich (consequently verging on mesotrophic, see Best and Traill, 1994) than those of the North. There have been a number of studies characterising the water chemistry of the loch (Best and Traill, 1994; Habib *et al*, 1997) and Table 1.4 summarises the difference between two representative sites, one in the North and the other in the South Basin.

Table 1.4: The Water Chemistry (selected parameters) of two representative sites in the North and South Basins of Loch Lomond (data from SEPA, West Region).

Parameter	North Basin at Inveruglas	South Basin at Creinch
Conductivity ($\mu s.cm^{-1}$)	44	62
Total Hardness (mg $CaCO_3.l^{-1}$)	11	18
Chloride ($mg.l^{-1}$)	6.5	8.0
pH	6.9	7.0
NH_3/N ($\mu g.l^{-1}$)	30	40
TON ($\mu g.l^{-1}$)	45	170
PO_4/P ($\mu g.l^{-1}$)	2	4

There have been few studies carried out so far on water circulation patterns in Loch Lomond with one exception being that of Curran and Poodle (1994). In that study, it was found that the inflow from the Endrick Water (the largest single inflow source of water and sediment) flows northwards between Inchcailloch and the mainland (see Fig 1.2) and current speeds as high as 0.13 $m.s^{-1}$ can be attained. In addition, water turbulence was measured and dilution rates consequently calculated. It was found that at Midross (a comparatively open water site on the western shores of the South Basin) dilution rates of 30 fold could be expected within 1.5 hours.

In another study (Murphy *et al.*, 1994), in which the aquatic macrophyte assemblages of the loch were analysed, wave exposure indices were calculated, with the greatest values occurring at south west facing shorelines of the South Basin, consistent with that expected from prevailing wind direction (see Figs. 1.1 and 1.2) and the exposed nature of the South Basin.

The planktonic communities of Loch Lomond are influenced by the contrasting water chemistry regimes of the North and South Basins of the loch with greater abundance and diversity of both phytoplankton and zooplankton found in the South Basin (see Habib *et al.*, 1997; Habib, 1993). The fish community of the loch and the considerable changes it has undergone is reviewed in Adams (1994) while the macrophyte assemblages are described in Murphy *et al.* (1994).

1.4.2: Tourism Pressure on Loch Lomond

Loch Lomond, by virtue of its location near the heavily populated Central Belt, is subject to some of the most intense powerboat activity in Scotland. It is estimated that 2.6 million people live within a one hour journey of Loch Lomond by car or public transport (Hamilton, 1988). In addition, many tourists from other countries are attracted to the area. This gives rise to concern, both local and by tourists themselves about potential adverse effects of powerboating. For example, a recent tourist survey (East Loch Lomond visitor survey, 1989) has shown that a majority of the public were in favour of introducing controls on powerboating. Such concern led to the implementation of bylaws by the Loch Lomond Park Authority in 1995, to control boat activity (particularly powered craft) and consequently improve safety and the amenity value of Loch Lomond. However, as yet, no controls on the types of motors used are specified.

The recreational craft using Loch Lomond have been censused since 1989. These studies have shown that boat numbers are increasing on Loch Lomond (Adams and Grant, 1997; 1998). In addition to this, very high boat densities (about twelve times the mean for the whole loch) occur locally, particularly in some sheltered areas among the islands in the South Basin of the loch (see Adams *et al.*, 1992). In these areas of locally high boat density, there is an increasing risk of chemical pollution from powerboat HC emissions. In addition, the pattern of craft use on Loch Lomond is changing, with an increasing proportion of fast powered craft (which have the potential to cause the most ecological damage) being used (Adams and Tippett, 1994; Adams and Grant, 1997)

1.5: Aims of The Present Study

General Aim:

The general aim of the present study is to assess the risk of water pollution occurring in Loch Lomond as a result of powerboat operation.

Particular Aims:

1) To ascertain the approximate scale of powerboat HC emissions into Loch Lomond.
2) To chemically characterise outboard motor exhaust-polluted water (EPW).
3) To develop an appropriate method for the reliable detection and precise quantification of powerboat exhaust HCs in freshwaters.
4) Using the method outlined in Aim 3),
   i. To investigate the temporal and spatial distribution of powerboat exhaust HCs in Loch Lomond in summer.
   ii. To examine the fate of powerboat exhaust pollutants in controlled experiments.
5) To assess the aquatic toxicity of outboard motor EPW. Two species were used:
   i. *Daphnia magna*, an international standard test organism.
   ii. *Diaptomus gracilis*, a copepod, native to Loch Lomond.
6) To attempt to define a theoretical environmentally safe concentration of powerboat exhaust pollutants.

Chapter 2

Powerboat Pollution: Estimation of the Discharge rate into Loch Lomond

2.1: Introduction

Total catchment modelling of discharge rates of pollutants is widely used to estimate the levels and potential impacts of nutrient pollutants (e.g. Gunn *et al.*, 1994; Broads Authority 1997). However, a modelling approach of estimating the scope for water pollution from powerboat exhaust has only been carried out in two previous related studies (Stolpe, 1992; Mele, 1993). In the latter study, the total annual hydrocarbon input from powerboats was estimated for all coastal and inland waterways over the whole of the United States. Only a rough estimate of hydrocarbon (HC) discharge could be gained, as many parameters involved in that estimate were themselves estimated.

In Stolpe's (1992) study, boat fuel consumption surveys were carried out near Barnegat Bay, New Jersey, an area subjected to heavy recreational boating pressure. Hydrocarbon input for Barnegat Bay was estimated using data from the fuel consumption surveys and previous studies in which the ratio of fuel consumed to hydrocarbons (HCs) exhausted from boat engines was measured.

In only one previous study (see Bartlett, 1989) has the total annual HC input been estimated for a single freshwater lake. Although details are lacking, it was stated that the total amount of HCs entering the Bodensee (Lake Constance) in 1977, including the input from freight traffic, was 30.16 tonnes.

Here, I construct a simple mathematical model from a combination of published and derived data, to estimate the annual HC input to Loch Lomond in one year for which detailed information on craft use is known (1989-90; see Adams *et al.*, 1992). In addition, also using published information (see Adams and Tippett, 1994; Bannan *et al.* 1995; Adams and Grant, 1998), changes in HC loading over time are estimated (1989-97).

2.2: Methods

To estimate the total annual hydrocarbon (HC) input for Loch Lomond, several variables of boats and their use were required:

(1) Boat numbers and types using Loch Lomond.

(2) The mean daily number of powered boats of each type in use on Loch Lomond throughout the year.

(3) Mean engine sizes and types for each powered boat type on Loch Lomond.

(4) HC emission rates for each engine type.

(5) The mean daily operational time period for boats on Loch Lomond.

2.2.1: Boat Numbers and Categories on Loch Lomond

2.2.1.1: Boat Census Methods

Boat censuses on Loch Lomond have been carried out on a number of days each year since 1989, using an identical protocol. Boats were counted visually by two observers, from a fast moving speedboat as it travelled over a pre-selected route during the period of peak boating activity, between 12 noon and 4 p.m. (see Fig. 2.1). Estimates suggest that at least 70.5 km^2 (over 99 %) of the surface area of the loch is visible from this transect route (see Adams *et al.* 1992). During the surveys, each boat was categorised as belonging to one of 12 previously identified boat types (see Table 2.1) and classified as being moving or temporarily stationary (but in use on that day). To examine the spatial distribution of craft on the loch, boats were counted separately in 12 geographical areas along the transect route. In a large-scale survey carried out in 1989-1990, 84 counts were performed throughout the course of one calendar year, with seven per month: five on weekdays and two on weekend days (see Adams *et al.*, 1992).

More limited surveys than that of 1989-1990 have taken place since then. In these subsequent surveys, nearly all of the censuses were carried out on summer weekend days, to focus survey effort on the busiest days of the year (ascertained from the 1989-1990 survey).

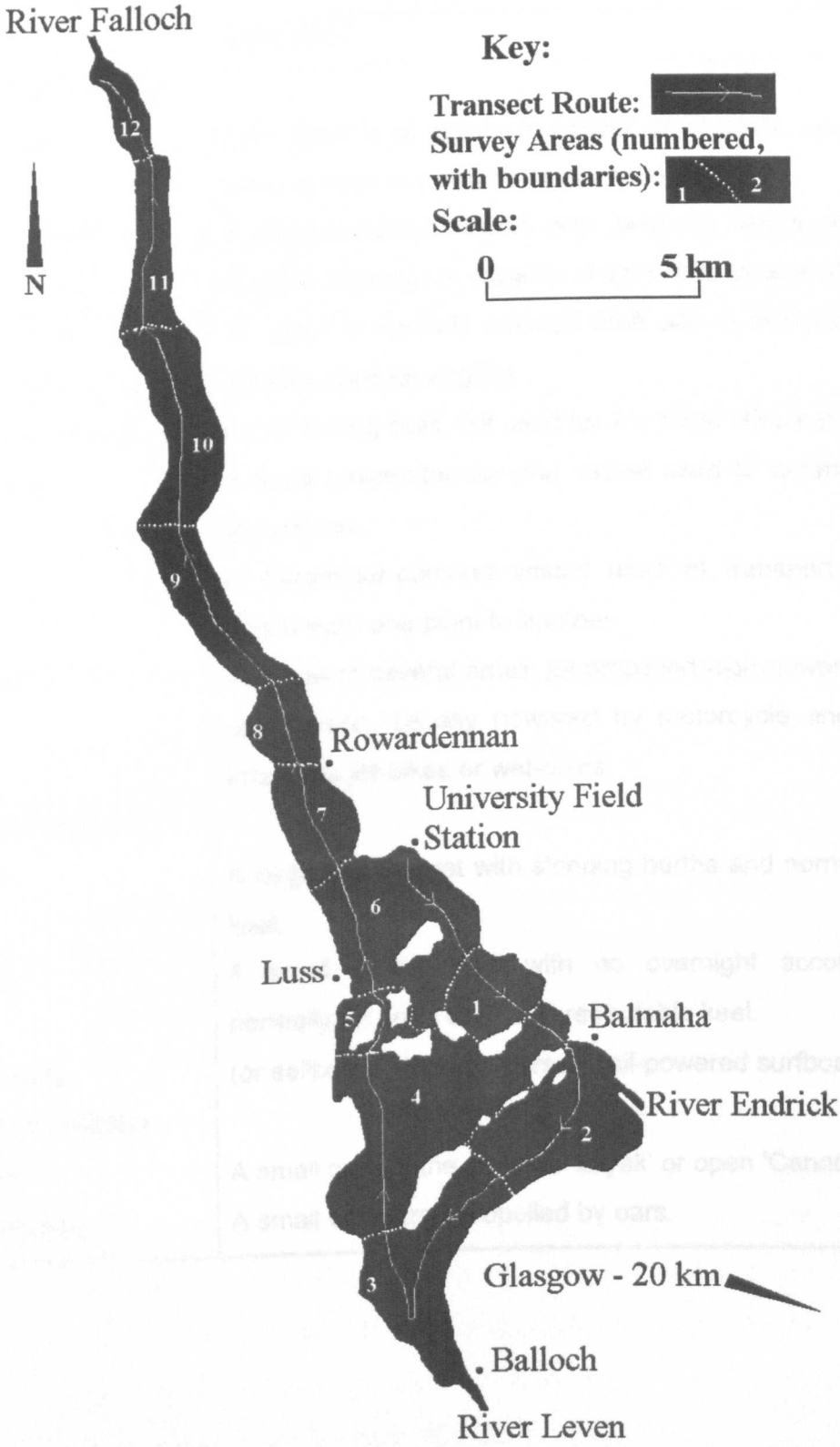

Fig. 2.1: *Map of Loch Lomond, showing the twelve boat census survey areas and the route (dashed line) taken by the survey boat.*

Table 2.1: Craft types using Loch Lomond.

Class	*Description*
POWERED CRAFT	
Speed-boat	Craft capable of speeds in excess of 20 knots, usually with a 'planing'-type hull and no sleeping berths.
Motor-cruiser	A displacement-hull craft with sleeping berths and inboard engines, usually not capable of speeds in excess of 20 knots.
Fishing boat	An open or partially covered craft with a displacement hull, actively used for angling.
Pleasure day-boat	As for fishing boat, but used for any other leisure pursuit.
Tour boat	A large passenger-carrying vessel used to organise circular excursions.
Ferry	A passenger-carrying vessel used to transport people or goods from one point to another.
Jet-ski	Any one of several small, jet-propelled high-powered craft for one person, usually powered by motorcycle engines. Also known as jet-bikes or wet-bikes.
SAILING CRAFT	
Yacht	A large sailing boat with sleeping berths and normally a fixed keel.
Dingy	A small sailing craft with no overnight accommodation, normally an open deck and retractable keel.
Windsurfer	(or sailboard) A single person sail-powered surfboard.
SELF POWERED	
Canoe	A small craft of the covered 'kayak' or open 'Canadian' type.
Rowing boat	A small open craft propelled by oars.

2.2.1.2: The Mean Daily Number of Operational Boats on Loch Lomond in Each Powered Boat Category, Throughout the Year.

In order to estimate the total annual HC input for Loch Lomond, data on the mean daily number of craft for each powered boat category using Loch Lomond were necessary. The most comprehensive data available is that from the 1989-1990 boat census survey (described in Adams *et al.*, 1992) and from this, monthly mean daily boat densities throughout the year can be calculated, for each powered boat category. All powered craft in use on a particular census day, including boats temporarily stationary, were counted in each census and included in the calculation of the total HC discharge into Loch Lomond. The mean annual powered boat density (number of boats per day) in each boat category may then be worked out, as the mean of the twelve monthly mean densities.

2.2.2: Engine Sizes and Types for Each Boat Category.

Data on the mean numbers of each type of powered boat on Loch Lomond in themselves are inadequate for the calculation of the loch's annual HC input. Some categories of boats, such as cruisers and speedboats, may be propelled by different engine types, which have different pollutant emission rates. To account for this, the mean annual number of operational boats were calculated separately for each boat and engine type.

Engine sizes and types for each craft category were determined during a survey, carried out on Loch Lomond on August 21 1994. One assumption of the model is that the mean engine sizes on the loch did not change significantly between 1989 and 1994. Engine types and sizes were determined for all powered boats actually in use on Loch Lomond on the census day. Five types of engines were observed:

- Two-stroke outboard motors
- Four-stroke outboard motors

- Petrol powered inboard engines
- Diesel powered inboard engines
- Jet-ski engines

Actual engine size (in horsepower, hp) of each craft was determined by a visual inspection of the engine identification plate, on which the rated power output (in hp) is printed.

2.2.3: Calculation of Hydrocarbon Emission Rates for Each Combination of Engine and Boat Type.

The rate of hydrocarbon emissions from an engine depends on its type, its throttle setting, its power rating (in hp) and the amount of time the engine is operated. Coates and Lassanske (1990) produced hydrocarbon (HC) emission figures for a 70 hp two-stroke outboard engine (the mean size of this engine type in the USA at the time), operated at a range of throttle settings, for time intervals deemed to approximate normal everyday use (see Table 2.2). On the throttle setting regimes in that study, the motor was found to emit 1529 g of HC material per hour on average, in addition to 2100 g of carbon monoxide (CO) and 31 g of nitrous oxides (NOx).

Table 2.2: HC emissions from a 70 hp engine, at a range of throttle settings. Data from Coates and Lassanske (1990).

% of Rated Speed	Torque - % Full Throttle	% of Time Operated at this Throttle Setting	Total HC Emissions ($g.hr.^{-1}$)
100	100	6	5360
80	71.6	14	2751
60	46.5	15	1812
40	25.3	25	1260
Idle	0	40	589
Weighted Average: all speeds	Weighted Average: all throttle settings	Operated at all settings	1529

A study by the Boating Industry Associations (BIA, 1975) is one of the few in which the emissions (in this case, the total volume of exhaust gases) of a wide range of two-stroke outboard motors of different sizes were measured. In that study, engines ranging from 3.6 to 105 hp were used. As in the study by Coates and Lassanske (1990), a range of throttle settings were used, and the mean emission quotient of this range of settings (simple arithmetic mean) is shown in Fig 2.2 for each engine. For the calculations presented here, it was assumed that the emission rates of engines in the present study that were larger than 105 hp could be determined by extrapolation of the regression of emission rates on engine size of all of the smaller engines in the BIA (1975) study (Fig 2.2).

For each different engine type on Loch Lomond, the mean power output was calculated (from the August 1994 survey) and its emission quotient was estimated using the regression equation of emission rate on engine power. This was done, firstly by estimating HC emissions by the 70 hp engine used by Coates and Lassanske (1990) which had already been accurately measured by a different method and found to be 1529 g of hydrocarbons (HCs) per hour by the BIA (1975) method. Using that study, emissions of this engine were estimated at 74.70 cubic metres per hour. One of the assumptions of the current study is that the 74.70 m^2 of gases emitted hourly, as calculated for a 70 hp engine in the BIA (1975) study equated to the 1529 g of HCs emitted per hour from an identically powered engine, measured in the Coates and Lassanske (1990) study. To calculate the mean quantity of emissions from each engine type using Loch Lomond, the relationship in Fig. 2.2 was used, with emission rates converted from cubic metres of gaseous emissions to grams of HCs, by using a conversion factor (20.47), derived from the comparison of emission rates for a 70 hp engine in each of these two studies. Thus, a second assumption of the present study is that the quantity of HCs per unit volume of total gaseous emissions remains constant over a range of engine sizes.

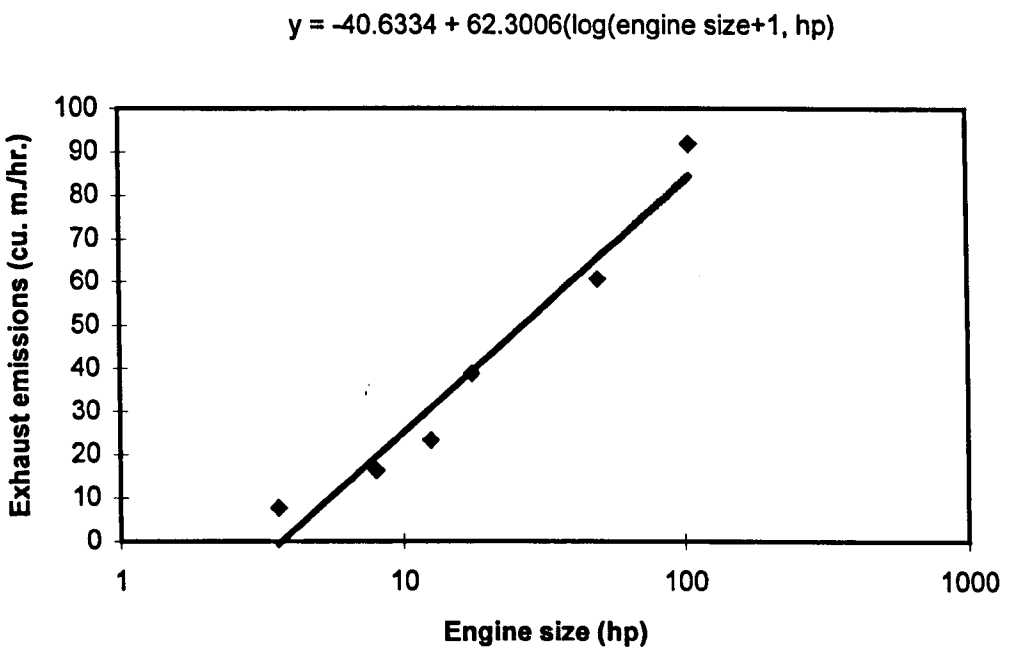

Fig. 2.2: The relationship between engine size (displayed on a logarithmic scale) and quantity of emissions (data from the BIA, 1975). This relationship is described by the equation: y = -40.63 + 62.30(log(x+1)), whereby:
y represents exhaust emissions, measured in cubic metres of exhaust gases per hour.
x represents engine size in hp.

According to Mele (1993), four-stroke petrol inboard and outboard engines emit approximately 7 % of the HC material of a two-stroke outboard with equivalent power output. In the current study, emission rates for these engines were calculated as a 7 % proportion of the emissions from a similarly powered two-stroke engine.

Mele (1993) states that diesel 4-stroke boat engines are considerably less polluting than the corresponding petrol engines. In another study, (Jemma *et al.*, 1995), it was found that in the case of 4-strokes car engines, diesel engines produce only 35% of the amount of HC material as petrol engines. For the present study, it was assumed that similar relative efficiencies pertained to diesel and petrol 4-stroke boat engines and diesel boat engine emission rates were calculated as a 2.45 % proportion of the emissions from a

similarly powered petrol two-stroke boat engine (i.e. 35 % of the amount emitted by a petrol four-stroke boat engine).

Jet-ski engines closely resemble those of large two-stroke motorcycles in design. In two previous studies (Jemma *et al.*, 1995; Chan *et al.*, 1995), the HC emissions of two-stroke motorcycles were measured. They were found to be highly polluting, producing 1.5 times as much HC material as a two-stroke outboard motor with the same power output. However, the aforementioned motorcycle engines had a much smaller rated power output than the jet-skis on Loch Lomond. To estimate a discharge rate for these jet-skis, it was assumed that the relationship between engine size and HC emissions for two-stroke outboard engines could be applied to motorcycle and jet-ski engines. As the latter are more polluting, their emission rates were calculated by multiplying those of similarly powered two-stroke engines by a conversion factor of 1.5.

2.2.4: The Mean Daily Operational Time Period for Boats on Loch Lomond

Mele (1993) found that the mean daily period of operation for recreational powerboats in the United States was three hours. It was assumed that a similar period of operation would also apply to powerboats on Loch Lomond.

2.2.5: Trends in the Annual HC Input for Loch Lomond, 1989-1997.

To gain an indication of any trends of the annual HC input into Loch Lomond, mean weekend summer (June to September) powerboat numbers from 1990 to 1997 (censuses from May 1990 onwards were carried out almost exclusively on summer weekend days) were compared. From the results of the 1989-1990 survey, it was found that mean weekend day summer boat densities were 5.204 times higher than the mean daily boat densities (including all weekdays and weekend days) for the whole year. To

calculate the annual discharge rates for 1989 to 1997, it was assumed that this ratio remained constant over this time period.

2.3: Results

2.3.1: Boat Numbers and Categories on Loch Lomond

Mean daily numbers (over all days, including weekdays and weekends) of powered boats of each type (see Table 2.1) for the one year period from March 1989 to February 1990 in Loch Lomond are shown in Table 2.3. As can be seen, day boats (including fishing boats) were the most common boat type observed during this period.

Table 2.3: Mean daily numbers of powered boats active on Loch Lomond during the boat survey of 1989-1990. The day boat category includes fishing boats and the tour boat category includes ferries.

Boat category	Mean daily number throughout the year, including weekdays and weekend days
Speedboat	11.82
Cruiser	11.45
Day boat	12.90
Tour boat	1.62
Jet-ski	0.71

2.3.2: Engine Sizes and Types for Each Boat Category.

The results of the engine size survey of August 21 1994 are presented in Table 2.4. Data from this survey were also used to estimate the mean daily numbers of boats in use for each boat and engine category, throughout the time period of the 1989-90 survey.

It was assumed that the proportions of the different engine types within boat categories remained constant from 1989 to 1994.

Table 2.4: Mean boat engine sizes for each boat and engine category, with the range of sizes also indicated (data from the survey of August 21 1994). The mean daily numbers of boats in use for each boat and engine type using Loch Lomond in 1989-1990 is also shown, estimated from the 1994 engine size survey.

Boat type	*Engine type*	*Mean engine size (hp)*	*Range of engine size (hp)*	*Estimated mean daily numbers of boats in use, 1989-90*
Speedboat	Outboard	90.3	20-200	8.87
	Inboard Petrol	242.7	140-1500	2.95
Cruiser	Inboard Petrol	233.4	20-600	9.59
	Inboard Diesel	155.6	30-400	1.86
Tour/Ferry	Inboard Diesel	93.1	25-180	1.62
Day-boat	Outboard	7.9	2-15	12.90
Jet-ski	Motorcycle	58.8	30-60	0.71

2.3.3: Hydrocarbon (HC) Emission Rates for Each Engine and Boat Type.

Using the regression of volumetric emission rate versus engine size relationship (calculated in section 2.2.3), the assumed HC weight equivalent emission rates for each boat and engine type are estimated (see Table 2.5). Emission rates are calculated thus:
Emission rate (in grams of hydrocarbons [HCs] per hour) = 1275 x ($\log_{10}$(engine size, hp + 1)) - 832
For inboard petrol engines, inboard diesel engines and jet-skis, the values gained are then multiplied by correction factors of 0.07, 0.0245 and 1.5 respectively, to account for the different HC emission rates of these engine types (see section 2.2.3).

As can be seen, jet-skis have the highest emission rates, producing an estimated mean (Loch Lomond craft) of 2150 g of HC per hour, closely followed by speedboats powered by two-stroke outboard motors.

The estimated range of emission rates for each boat engine category is also indicated, based on the range of engine sizes. The emission rate of the smallest day boat engines could not be calculated, as they have a power output ranging as low as 2 hp. This is smaller than the smallest engine (3.6 hp), for which emissions were measured in the BIA (1975) study and application of the above formula yields a negative quantity of engine emissions. This did not present a problem in the overall calculation, as the mean day boat engine size was 7.9 hp (considerably larger than the smallest engine in the study by the BIA, 1975), for which emission rates are clearly calculable.

Table 2.5: HC emission rates for each boat engine type on Loch Lomond. The mean and range are given both for boat engine sizes and emission rates.

Boat Type	*Engine type*	*Mean engine size (hp)*	*Range of engine size (hp)*	*Mean HC emissions per hour, per engine (grams)*	*Range of HC emission rate (grams)*
Speedboat	Outboard	90.3	20-200	1668	854-2105
	Inboard Petrol	242.7	140-1500	155	134-225
Cruiser	Inboard Petrol	233.4	20-600	153	60-190
	Inboard Diesel	155.6	30-400	48	26-61
Tour/Ferry	Inboard Diesel	93.1	25-180	41	24-50
Day-boat	Outboard	7.9	2-15	378	up to 703
Jet-ski	Motorcycle	58.8	30-80	2150	1604-2402

2.3.4: Total Annual HC Input for Loch Lomond in 1989

Combining all of the relevant parameters (including the mean daily operational time period per boat of three hours) gives a figure for the total annual input of HCs into Loch Lomond, shown in Table 2.6, of 25.50 tonnes (based on 1989 boat census data). As can be seen this mostly (63.5 %) consists of emissions from speedboats powered by outboard motors. A further 21.0 % was produced by day-boats powered by outboard motors. Jet-skis contributed 6.5 % of the total and cruisers powered by petrol inboard motors 6.3 %. Cruisers and tour boats with diesel engines between them contributed only 0.7 % of the total.

Table 2.6: Total annual HC discharge into Loch Lomond: Totals for each boat type and grand total (data derived from Adams *et al.*, 1992 BIA, 1975 and Coates and Lassanske, 1990).

Boat Type	*Engine Type*	*Annual HC input for each engine type (tonnes)*
Speedboat	Outboard	16.20
	Inboard Petrol	0.50
Cruiser	Inboard Petrol	1.61
	Inboard Diesel	0.10
Tour/Ferry	Inboard Diesel	0.07
Day-boat	Outboard	5.35
Jet-ski	Motorcycle	1.67

GRAND TOTAL: 25.50 TONNES

2.3.5: Long-Term Changes in HC Emissions to Loch Lomond

By applying similar techniques to those used to calculate engine emission totals for 1989, the annual HC discharge was calculated for the years 1990-1997. Fig. 2.3 and Table 2.7 both show the increase of this annual input since 1989. Using regression analysis, it was shown that this increase was highly statistically significant (i.e. with a gradient significantly >0; $t = 4.63$, $p < 0.01$, 8 d.f.). The overall increase is due to both an increase in boat numbers and also in the proportion of speedboats, powered by two-stroke outboard motors and jet-skis. These two boat types are by far the most significant emitters of HCs into Loch Lomond (Bannan *et al.*, 1995).

Table 2.7: The increase in HC input for Loch Lomond for each boat engine type from 1989 to 1997.

Boat Type	Engine Type	1989	1990	1991	1992	1993	1994	1995	1996	1997
Speedboat	Outboard	16.20	20.40	28.42	21.10	20.53	27.68	46.14	35.55	46.63
	Inboard Petrol	0.50	0.63	0.88	0.65	0.63	0.85	1.42	1.10	1.44
Cruiser	Inboard Petrol	1.61	1.01	1.60	0.67	1.30	1.34	2.46	2.30	3.11
	Inboard Diesel	0.10	0.06	0.10	0.04	0.08	0.08	0.15	0.14	0.19
Tour/Ferry	Inboard Diesel	0.07	0.04	0.07	0.03	0.08	0.08	0.15	0.11	0.14
Day-boat	Outboard	5.35	3.86	3.77	1.99	2.34	2.81	4.91	3.56	4.81
Jet-ski	Motorcycle	1.67	3.55	5.68	5.75	5.26	7.53	9.88	13.00	14.58
Total annual HC input (tonnes)		25.50	29.55	40.51	30.24	30.23	40.38	65.11	55.75	70.91

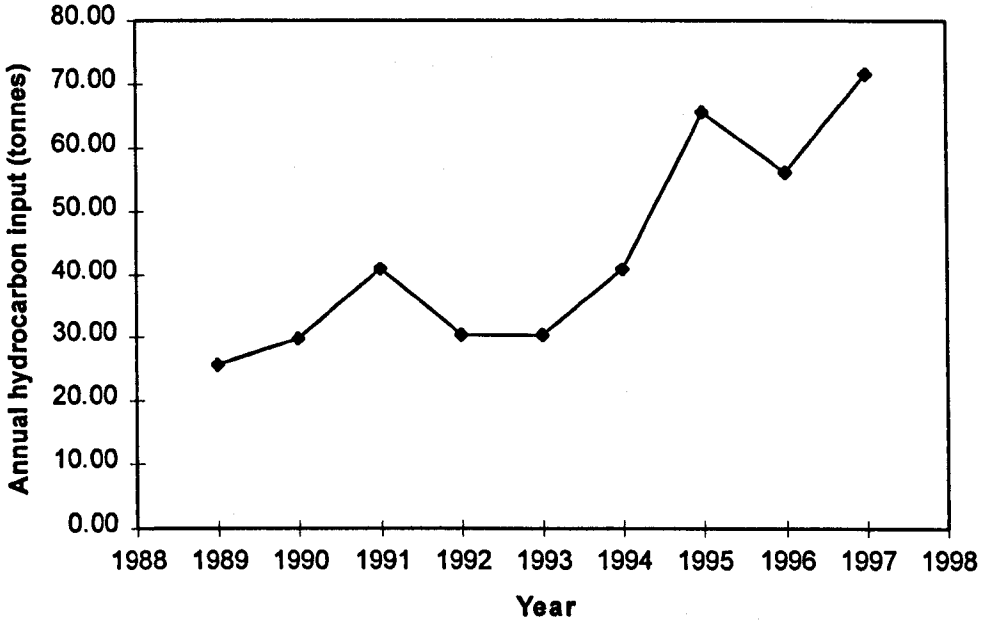

Fig. 2.3: The increase in the total input of HCs into Loch Lomond from 1989 to 1997. This increase is predicted by the equation:
$y = 5.33x + 10571$, whereby
y represents the annual HC input in tonnes
x represents the year.
This was a strong statistical relationship ($r^2 = 71.8\ \%$, $F_{1,8} = 21.42$).

2.4: Discussion

2.4.1: General Conclusion

The model presented in the current study utilises published and unpublished data on craft activity, engine sizes and size-related discharge rates to calculate the total annual discharge of hydrocarbons (HCs) into Loch Lomond for 1989, a year for which a comprehensive data set of craft censuses was available. In addition, the annual hydrocarbon (HC) loading for the years 1989 to 1997 was also calculated, using a similar modelling technique. The technique, along with all modelling methods, involved several assumptions. The validity of these assumptions is explored here. Despite the uncertainty of the accuracy of some of the parameters used in this model, it does provide the best estimate of the magnitude of the potential discharge (25.50 tonnes in 1989) and the probability that this has increased from 1989 to 1997. This increase resulted from increases in the numbers of speedboats powered by outboard motors and jet-skis. The proportions of these two engine types, both far more polluting than any other, are also increasing, reflecting a trend towards the use of craft that may cause the most environmental damage.

2.4.2: Assumptions in the Annual HC Input Calculation

2.4.2.1: Engine Emission Rates

Engine emission rates for the mathematical model for HC input calculation were derived from Coates and Lassanske (1990). However, the estimate of total HC discharge in the current study may be conservative. A study by the US Environmental Protection Agency (US EPA, 1991) stated that as much as 25 % of fuel used by two-stroke motors is exhausted unburned, a figure three times greater than the 8.3 % estimated by Coates and Lassanske (1990). It is also likely that old, worn engines emit substantially more HC

material than new engines (Mele, 1993). In addition, the figures for annual HC input into Loch Lomond presented here neglect fuel spillage. This may be possible to estimate, and could be significant in some areas, such as marinas and refuelling points.

An assumption that needed to be made is that the emission rates of engines of over 105 hp were governed by the relationship between engine size and HC emission rate described in Section 2.2.3. It is not certain if this relationship holds true for the largest engines on Loch Lomond, many of which exceed 105 hp in power output. Further research would be useful, to precisely measure the emission rates of these larger engines.

2.4.2.2: Daily Boat Operational Period

The daily operational time period of three hours is the assumed average time for which each boat is operated on Loch Lomond and was gained from a previous study (Mele, 1993) in which the average daily powerboat use in the USA was calculated. There is some evidence that this may be a conservative estimate of average daily use, at least in summer on Loch Lomond. In a survey of boat launching activity, carried out in August 1994, relatively high launching activity persisted for over five hours. This could correspond to a similar time period of heavy powerboat activity on the loch. If this five-hour period is incorporated in the annual HC input estimate, a much higher value (which could indeed be more accurate) would result.

2.4.2.3: HC Evaporation

In the model described here, the total amount of hydrocarbon (HC) emissions from powered boats has been estimated. However, many of these compounds are volatile and may evaporate immediately. Thus, this model may overestimate the total amount of HCs remaining in the water over a longer time period.

2.4.3: Solutions to the Problem

A feature of the total HC discharge estimate is the tremendous potential for its reduction, which may be achieved by various means. The most obvious method, evident from the discharge data in Table 2.5, is the reduction of the number of two-stroke outboard engines. Alternatives to this engine type exist for speedboats and day-boats. The former may be propelled by inboard engines, or gas-powered (inboard or outboard) engines which produce minimal HC emissions (Mele 1993). Recently, there has been another encouraging development. A new fuel injection system has been developed for 2-stroke outboard motors (Hamer, 1994) which reduces HC emissions by 75 %. Legislation could be introduced to enforce the use of these cleaner engines, as has been successfully done with car engines (Chan *et al*, 1995). Fishing boats (and some day-boats) could in future be powered by electric motors if the technology is properly encouraged, specifically the increase of their range which is still very limited. Indeed, further research in engine technology may improve the efficiency of all boat engines.

Fuel consumption of outboard motors can be as much as ten times that of automobile engines, given the same speed and power output (Mele, 1993) and the reduction of this figure would be of great benefit, not least to boat users themselves. Fuel consumption efficiency in boats is often overlooked, as powerboating, particularly operation of speedboats and cruisers, is a luxury activity, pursued by people with significant disposable income. Thus the price of large quantities of petrol may not be considered as significant.

Chapter 3

The Effects of Powerboat Exhaust on Water Chemistry

3.1: Introduction

3.1.1: General Introduction

3.1.1.1: Context of the Present Study

In the previous chapter, the total hydrocarbon (HC) discharge from powerboat exhaust into Loch Lomond was estimated. This discharge was found to be increasing. As a next step in assessing the risk to the environment, this chapter aims to determine the chemical effects of powerboat (particularly outboard motor) operation on water quality.

3.1.1.2: Previous Relevant Work

Previous work has shown that most of the compounds emanating from powerboat exhausts are hydrocarbons (HCs) from unburned fuel (see Montz *et al.*, 1982; Jüttner, 1994, 1995*a*). Some products of incomplete combustion of HCs, such as phenols and benzaldehydes (oxygenated derivatives of HCs) are also produced (Jüttner, 1994).

Besides hydrocarbons and their derivatives, powerboat engines also produce other pollutants. Lubricant oils may contain zinc and sulphur (Jackivicz and Kuzminski, 1973, Liddle and Scorgie, 1980). Engine operation can increase water temperature and conductivity, and the levels of carbon dioxide, sulphate and lead (see Boating Industry Associations, 1975). Lead has proved to be a useful indicator of powerboat pollution in previous studies (e.g. Byrd and Perona, 1980; Horsfall *et al.*, 1988). Recently, the increasing popularity of unleaded fuel has probably decreased the risk of lead pollution (Murphy *et al.*, 1993) but there are still some powerboat engines running on leaded fuel on Loch Lomond today.

It is known that increases in carbon dioxide cause corresponding increases in bicarbonate levels and decreases in pH in freshwater, consistent with the equilibrium reaction of carbon dioxide in water (Hutchinson, 1957):

$$CO_2 + H_2O \rightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^-$$

At low pH (about 4.4), the CO_2 is in the form of H_2CO_3. With increasing pH (from about 4.4 to 6.5), this equilibrium reaction is shifted to the right. H_2CO_3 (carbonic acid) then changes to $H^+ + HCO_3^-$ (bicarbonate ion), thus increasing bicarbonate levels.

Here, I attempt to chemically characterise artificially produced outboard motor exhaust-polluted water (EPW) particularly with respect to HC pollutants but also certain

basic physical and chemical parameters.

3.1.2: Determination of Effects of Powerboat HC Emissions on Water Quality

There are presently a wide variety of techniques available for the analysis of HCs in water, with some of these being more precise and specific than others. The principal analytical methods are reviewed in Appendix I (along with the reasons for choosing the methods for the current study). For the detailed analysis proposed here, Gas Chromatography-Mass Spectrometry (GC-MS) was chosen. This technique has two important attributes:

- individual compounds can be identified, making the technique specific to a huge variety of compounds (depending on column and mass spectrometer conditions), in the case of the present study, volatile aromatic HCs.
- the technique is highly sensitive and useful for detecting the very low concentrations of organic compounds which occur in environmental samples.

3.1.3: Aims

General Aim

- To assess the effects of outboard motor operation on water chemistry.

Specific Aims

1) To identify water-soluble and persistent HCs (both individual compounds and the total amount of HCs) and other exhaust contaminants, in polluted water.

2) To determine whether exhaust pollutants can be detected in a large lake (Loch Lomond) with heavy powerboating activity.

3) To compare the composition of the HCs in outboard motor EPW with those in outboard motor fuel: lubricant oil mixture, prior to combustion.

4) To investigate the effects of powerboating on certain physical and chemical parameters of freshwater: temperature, pH, bicarbonate, and oxygen levels.

3.2: Methods

3.2.1: Procedure of Water Sampling for Hydrocarbon Analysis

3.2.1.1: Preparation of glassware

Hydrocarbons have a widespread distribution in the environment (Connell and Miller, 1984) and thus, care was needed when taking and handling water samples. To avoid HC contamination, there are only two materials from which sampling vessels (in contact with the water sample) can be constructed: glass or PTFE (Poly Tetra Fluoro Ethene or TEFLON; see Gordon and Keizer, 1974). In the present study, glassware with ground glass stoppered lids was used to obtain, process and store samples. Before sampling, all glassware was thoroughly cleaned overnight in a hot "Decon" bath before rinsing four times in each of the following solutions:

- Tap water
- Distilled water
- Reagent grade acetone
- Reagent grade hexane

Following this, all glassware was dried for at least 1 hour in an oven at 60°C. This is standard procedure for washing of glassware prior to environmental analysis of HCs (Best and Dawson, 1993). Following drying, any glassware without lids (such as graduated cylinders) was covered with aluminium foil.

3.2.1.2: Sampling Procedure

Fig. 3.1 is a schematic representation of the procedure of water sampling and HC sample processing in the current study. Glass bottles were held carefully at the water surface and allowed to fill slowly, with no water deeper than about 2 cm taken. In the

controlled experiments, samples were collected from the water surface at a specified location, 10 cm away from the engine mounting, perpendicular to the wall of the tank onto which the engine was attached. Since only a limited number of samples could be analysed by GC-MS (due to equipment, time and cost constraints), surface water only was sampled, as previous research (Wachs *et al.*, 1992) has suggested that powerboat exhaust HC contaminants were most likely to be found there. Samples were allowed to de-gas and the lid was placed on tightly, taking care to prevent any air getting trapped, which could have allowed some of the more volatile HCs to evaporate into the air space.

3.2.1.3: HC Sample Processing

Some exhaust components may have the potential to adsorb to glass surfaces (Law *et al.*, 1988). In order to capture these HC compounds with the extraction solvent, sample processing was carried out in the same vessels used for sampling (see Fig. 3.1). The extraction and reduction involved DCM and necessitated the use of a fume cupboard (in which to carry out the process), as DCM is a potent eye irritant (Muir, 1977; Sigma-Aldrich Material Safety Data, 1995). The solvent samples were held in a water bath at 27±1°C and evaporation (and hence, reduction in volume) was facilitated by an air pump connected to a clean Pasteur pipette, blowing air onto the liquid surface of the sample. Such reduced sample extracts are very stable and can be stored in a refrigerator at below 4°C for up to one year before analysis (MEWAM, 1988; Pirie, *pers. comm.*).

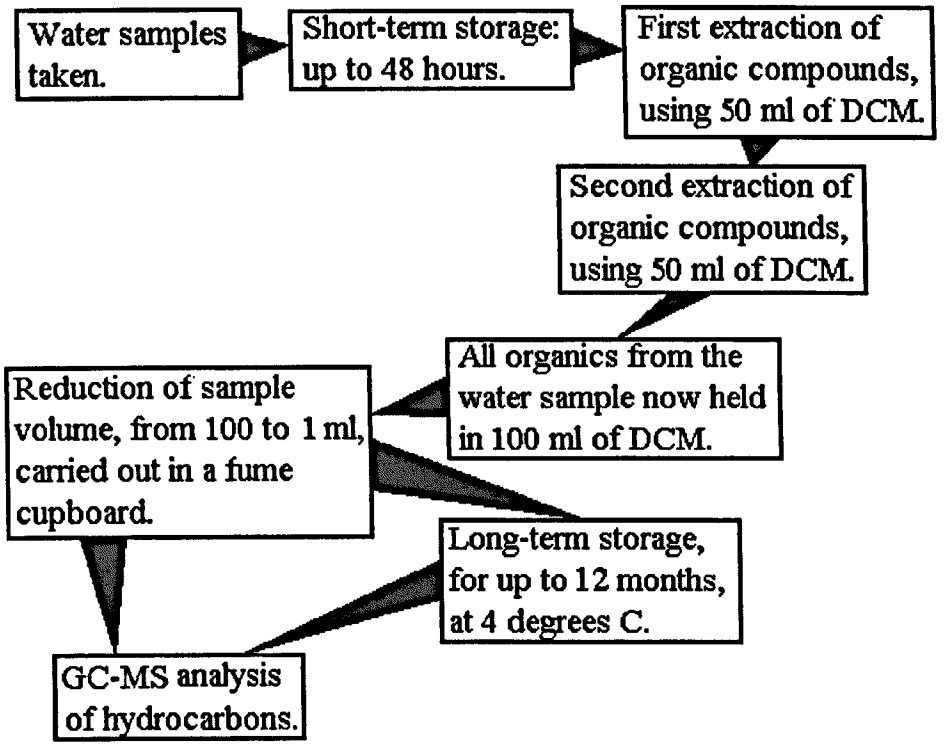

Fig. 3.1: *Schematic diagram, showing the hydrocarbon sample processing carried out in the present study.*

3.2.1.4: Hydrocarbon Analysis – GC-MS

Table 3.1: Summary of the GC-MS analytical conditions used in the present study.

A	Gas Chromatograph:		HP 5890
	Column:	Dimensions:	60 m x 0.25 mm. I.D.
		Material:	Fused Silica
		Stationary Phase:	DB-5
		Film Thickness:	0.25 μm
	Temperature Programme:	Initial Temperature:	30°C for 1 min.
		Programme:	6°C $min.^{-1}$
		Final Temperature:	300°C for 30 min.
	Injector:	Technique:	On-column
		Volume:	1 μl.
	Carrier Gas (moving phase):		Helium
	Carrier Gas Flow Rate:		1.2 $ml.min.^{-1}$
	Retention Gap:		2 m uncoated fused silica, 0.53 mm I.D.
B	Mass Spectrometer:	Specifications:	VG Trio 1
		Source Temperature:	200°C
		Interface Temperature:	300°C
		Electron Energy:	70 eV
		Filament Current:	150 μA
		Ion masses scanned:	50-500 a.m.u.

A: Capillary Column

A J&W DB-5 MS capillary column of length 60 m., internal diameter 0.25 mm., film thickness 0.25 μ with on-column injection was used for the separation process (or GC; see section A in Table 3.1). This means the entire 1 μl. of sample was injected into the column, which is the most appropriate technique for very dilute samples which typically occur in environmental analysis. The film in this case was a medium polarity liquid, appropriate for the separation of medium polarity compounds, such as aromatic hydrocarbons.

B: Mass Spectrometer (Identification of Chemical Constituents)

Mass Spectrometry (MS) operating conditions in this study are shown in Table 3.1, section B. The compounds were ionised by electron impact and of these fragments, all ion masses from 50 atomic mass units (a.m.u.), to 500 a.m.u. were monitored. The fragment pattern produced is diagnostic of a particular compound, and compounds were identified by comparing their fragment patterns with known fragment patterns stored in a computer database. Confirmation of identification is achieved by using appropriate standards.

3.2.1.5: GC-MS Compound Identification Procedure

The use of standards and library mass spectra is by far the most accurate way to identify a particular compound, although many compounds can be tentatively identified by using library spectra combined with retention times of the particular compound from previous studies using the same GC-MS operating conditions. This is most useful for those compounds most closely resembling the standards (such as isomers of them). Compatible GC-MS operating conditions to the present study were used in Wang *et al.* (1994), for the analysis of a light crude oil. Such an approach has also been used in a previous analysis of outboard motor EPW (Jüttner, 1994).

Other compounds could only be approximately identified, either to the nearest isomer (in the case of reasonably unambiguous mass spectra) or the nearest chemical group (for more ambiguous spectra). Table 3.2 shows the likely designations, with respect to identification of compounds detected in the chromatograms in the present study.

Table 3.2: The possible degrees of identification of peaks detected in chromatograms in the current study.

Method of identification	*Precision of identification*
a) Use of standards.	Definite.
b) Use of library spectra and known retention times from Wang *et al.*, 1994.	Tentative.
c) Use of library spectra alone (less ambiguous spectra).	Approximate (nearest isomer).
d) Use of library spectra alone (more ambiguous spectra).	Very approximate (nearest group of related compounds).
e) Unable to identify.	-

Combining and integrating each fragment pattern allowed the production of chromatograms of the whole sample, with each peak corresponding to a particular compound. Both Total Ion Chromatograms (TICs), combining all ion fragments scanned or partial ion chromatograms (PICs), only measuring particular ion fragments were created with detection limits of the TIC (based on the operating conditions presented in Table 3.1) being approximately 100 $ng.l^{-1}$ for the concentration of a particular chemical compound in water. The PIC is particularly useful in the identification of specific groups of compounds (Harriman, 1993), and these can be detected at far lower levels than in the TIC (Evershed, 1993).

To identify HC compounds in powerboat exhaust-polluted water, the standard used in the calibration of the GC-MS apparatus in the present study were chosen on the basis of data from two previous studies (Montz *et al.*, 1982; Jüttner, 1994). All of the chosen aromatic compounds (including 4-methyl-phenol, a phenolic compound) were among the most abundant exhaust pollutant compounds found in these studies. Three aliphatic HC compounds, n-hexane, n-octane and n-hexadecane, were also chosen, in order to briefly investigate these compounds in powerboat exhaust. The former two are known to be present in petrol (Johansen *et al.*, 1983) while the latter is known to be present in lubricant oils (Bartlett, 1989). Table 3.3 lists all of the standard compounds and their physical characteristics:

Table 3.3: Characteristics of the compounds used in this study as standards (data from Stephen and Stephen, 1963; Weast, 1988; Schwarzenbach *et al.*, 1993; Peterson, 1994).

Compound	Chemical Group	Chemical Formula	Molecular Weight	Maximum Water solubility ($mg.l^{-1}$)	Melting Point (°C)	Boiling Point (°C)	Relative Density ($g.cm.^{-3}$)
Benzene	Volatile Aromatic HC	C_6H_6	78	1780	+5.5	+80.1	0.877
n-Hexane	Aliphatic HC	C_6H_{14}	86	13	-95.0	+69.0	0.660
Methyl-benzene (Toluene)	Volatile Aromatic HC	C_7H_8	92	515	-95.0	+110.6	0.867
1,4 Dimethyl-benzene (p-xylene)	Volatile Aromatic HC	C_8H_{10}	106	200	+13.3	+138.3	0.861
4-methyl phenol (p-cresol)	Phenolic	C_7H_8O	108	19,400	+34.8	+201.9	1.018
n-Octane	Aliphatic HC	C_8H_{18}	114	2.28	-56.8	+125.7	0.703
1,3,5 Trimethyl-benzene (mesitylene)	Volatile Aromatic HC	C_9H_{12}	120	53	-44.7	+164.7	0.865
Naphthalene	Polycyclic Aromatic HC (PAH)	$C_{10}H_8$	128	35	+80.5	+218.0	0.963
Hexadecane	Aliphatic HC	$C_{16}H_{34}$	226	0.0036	+18.2	+287.0	0.773

To produce the standard, dilutions of each compound of 50 ppm (v/v) in DCM were made using a clean 50 µl "SGE" micro syringe, previously rinsed four times in each of the following solutions:

- 2 % "Decon 90" in tap water
- distilled water
- reagent grade acetone
- analytical reagent grade DCM.

In addition to the standard, the analytical grade DCM itself was analysed as a reagent blank.

3.2.2: Production of Outboard Motor Exhaust-Polluted Water

A "Mariner" 10 horsepower two-stroke outboard motor, running on a 50:1 mixture of "Thames" leaded petrol and "Castrol 2-stroke Motorcycle Oil" was used to produce water, contaminated by outboard motor exhaust. The motor was attached to a heavy wooden bracket which, in turn was securely bolted onto a concrete tank of 510 l capacity. The tank was then filled with untreated water from Loch Lomond using the University Field Station's experimental supply, taken from a depth of 2 m, at Ordnance Survey Grid Reference NS 377 957. The engine was run for a period of 60 minutes at 40% throttle (2420-2850 rpm, estimated from the manufacturers manual).

Prior to running the engine, a water sample was taken for HC analysis, to make sure that aqueous HC concentrations in the water used to fill the tank were not significant compared to the intentionally polluted water to be produced. Following engine operation, a sample was taken, to analyse the organic compounds in polluted water.

3.2.3: Analysis of Fuel:Oil Mixture, Prior to Combustion

A fuel:oil mixture (50:1 petrol:oil mixture, used to propel the engine used in the present study and a typical ratio used by outboard motors in general) standard was also produced, by diluting 10 μl of fuel, measured with the micro syringe, in 10 ml of dichloromethane. This gave a concentration of 0.1 % or 1 ppt (part per thousand), v/v.

GC-MS analysis was carried out using the conditions in Table 3.1. The possible degrees of accuracy of identification for each of the HC compounds in the fuel:oil mixture are the same as those in all of the water samples and are indicated in Table 3.2.

3.2.4: Analysis of powerboat exhaust pollutants in Loch Lomond

Fig. 3.2 shows a map of Loch Lomond with the position of five water sampling sites. Water samples for HC analysis were taken from three of these sites (A, B and C),. Of these three sites, site A (Balmaha Pier) is in an area with a large numbers of moorings and extensive servicing of boats, while Site C (North Narrows) is located in an area which has consistently shown the highest boat density in Loch Lomond with a mean of eight times that of the whole loch (see Adams *et al.*, 1992; Adams and Grant, 1997). For comparison, a third site, subject to low levels of boat activity was also chosen (Site B). That area consistently had a relatively low mean boat density of about half of that of the whole loch (Adams *et al.*, 1992; Adams and Grant, 1997) and the sampling site itself was located at a stretch of open water with relatively low boat traffic compared to that of the rest of the boat census survey area in which it is located (see Adams *et al.*, 1992, for more details of local boat densities at these three sites).

The other two sites (D and E in the map in Fig 3.2) were included in a programme of water sampling for basic physical and chemical parameters (see Section 3.2.5). Sites D and E were situated at locations subjected to levels of powerboat activity intermediate between that of Site B and that of sites A and C.

On Loch Lomond, surface water samples were collected from sites A, B, and C for HC analysis on two occasions:

1) on a weekday in winter (Thursday January 20, 1994), when boat densities are close to the lowest levels of the year, with almost no operational boats on the loch (Adams *et al.*, 1992).
2) on a weekend day in summer (Sunday August 7 1994), at 5 p.m., two hours after peak boating activity on that particular day.

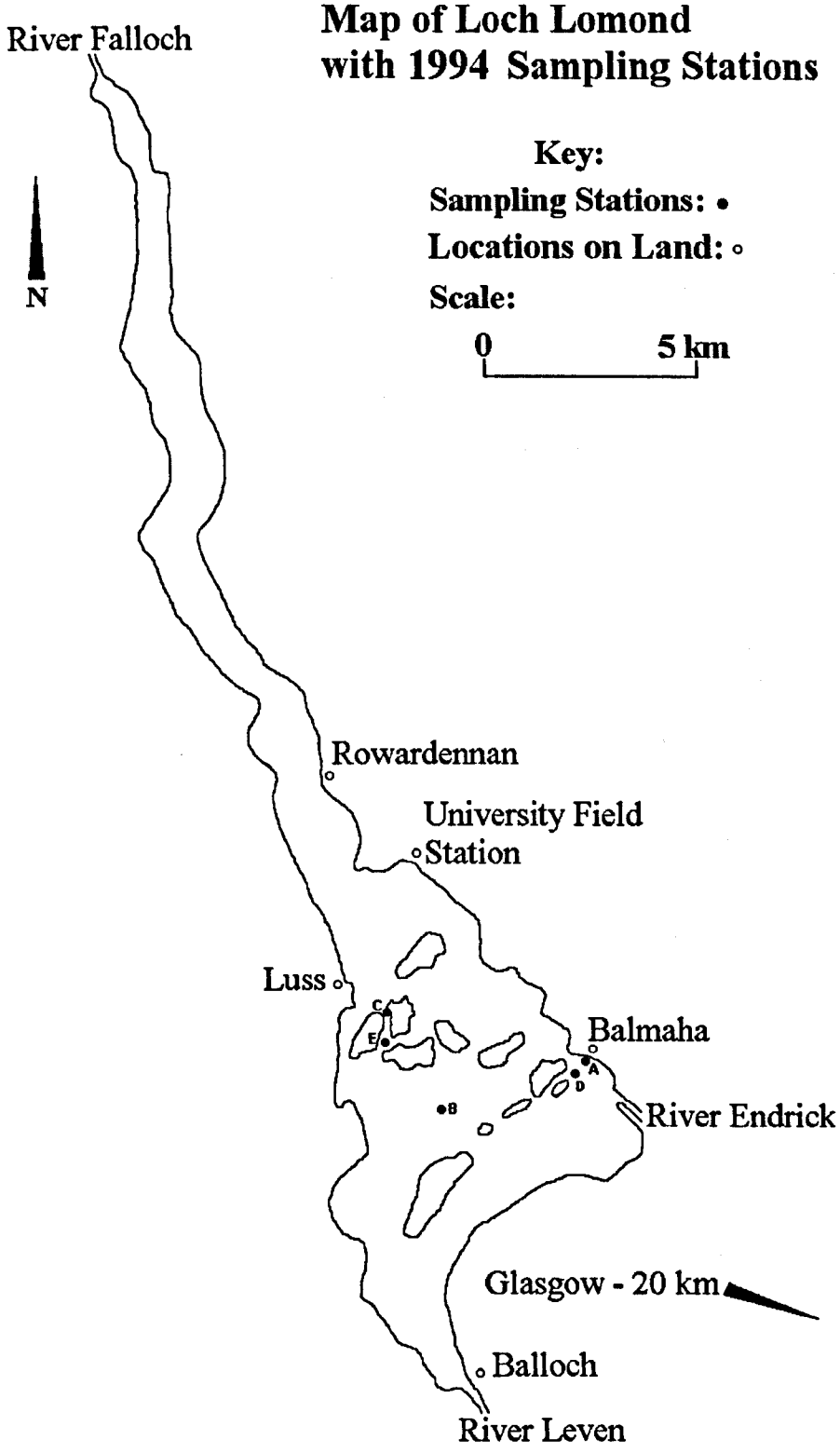

Fig. 3.2: Map of Loch Lomond showing the surface water sampling sites in the current study for 1994.

3.2.5: Basic Physical and Chemical Parameters

3.2.5.1: Controlled Experiments

Table 3.4 shows the times at which all samples for all chemical analyses were taken from the outboard motor tank during the course of four engine trials, carried out in December 1993, April 1994, July, 1994 and November 1994. One series of toxicity tests (using the copepod, *Diaptomus gracilis*) was run concurrently with the chemical analyses and samples taken for these toxicity tests are also indicated (see Ch. 6, Section 6.2.8 for more details of these tests).

Table 3.4: The times at which water samples for analysis of basic physical and chemical parameters were taken from the experimental tank, during four engine trials. The time interval shown is that elapsed after the running of the engine. The symbols indicate that a sample was taken at a particular time interval, with "*" representing samples for HC analysis, "•" representing samples for analysis of basic physical/chemical parameters and "t" representing samples for toxicity testing. The time interval of zero corresponds to the samples taken immediately after running the engine.

Time interval	Dec. 1993	Apr. 1994	Jul. 1994	Nov. 1994
Before engine operation	•	•*t	•	•*
Zero	•	•*t	•	•*
2 hours	•	•	•	•
6 hours	•	•	•	
1 day	•	•	•	
2 days	•	•	•	•
3 days	•	•	•	•
4 days	•	•	•	
5 days	•	•*t	•	•*
6 days	•	•	•	•
7 days	•	•	•	
8 days	•	•	•	•
9 days		•	•	
10 days		•	•	
11 days		•	•	•
12 days	•	•	•	•
43 days		•t		

3.2.5.2: Field Experiments

In addition to the laboratory experiments, the same basic physical and chemical parameters were analysed in surface water samples taken monthly, throughout 1994 from all five sites in Loch Lomond, indicated in Fig. 3.2.

3.2.5.3: Actual Sampling Procedure

All surface water samples for bicarbonate and pH analysis were taken using 1l. plastic sample bottles, using standard procedures (as described in Habib *et al.*, 1997). Prior to sampling, these bottles were thoroughly cleaned by an overnight bath in hot Decon 90, followed by rinsing in tap water and distilled water and finally air-drying. The surface water samples were then taken by holding the open bottles carefully at the water surface and allowing them to fill slowly. As with the HC analysis, no water deeper than about 2 cm was taken. Bicarbonate levels were measured on the day of sampling by titration against standardised 0.01M HCl, while pH was measured using a "Gallenkamp pH stick". Surface water oxygen levels and temperature on Loch Lomond and in the outboard motor tank were measured *in situ*, using a pHOx 62 TE temperature/oxygen probe.

3.3: Results

3.3.1: Hydrocarbon Analysis of Exhaust-Polluted Water (EPW)

3.3.1.1: Identification of Organic Compounds in EPW

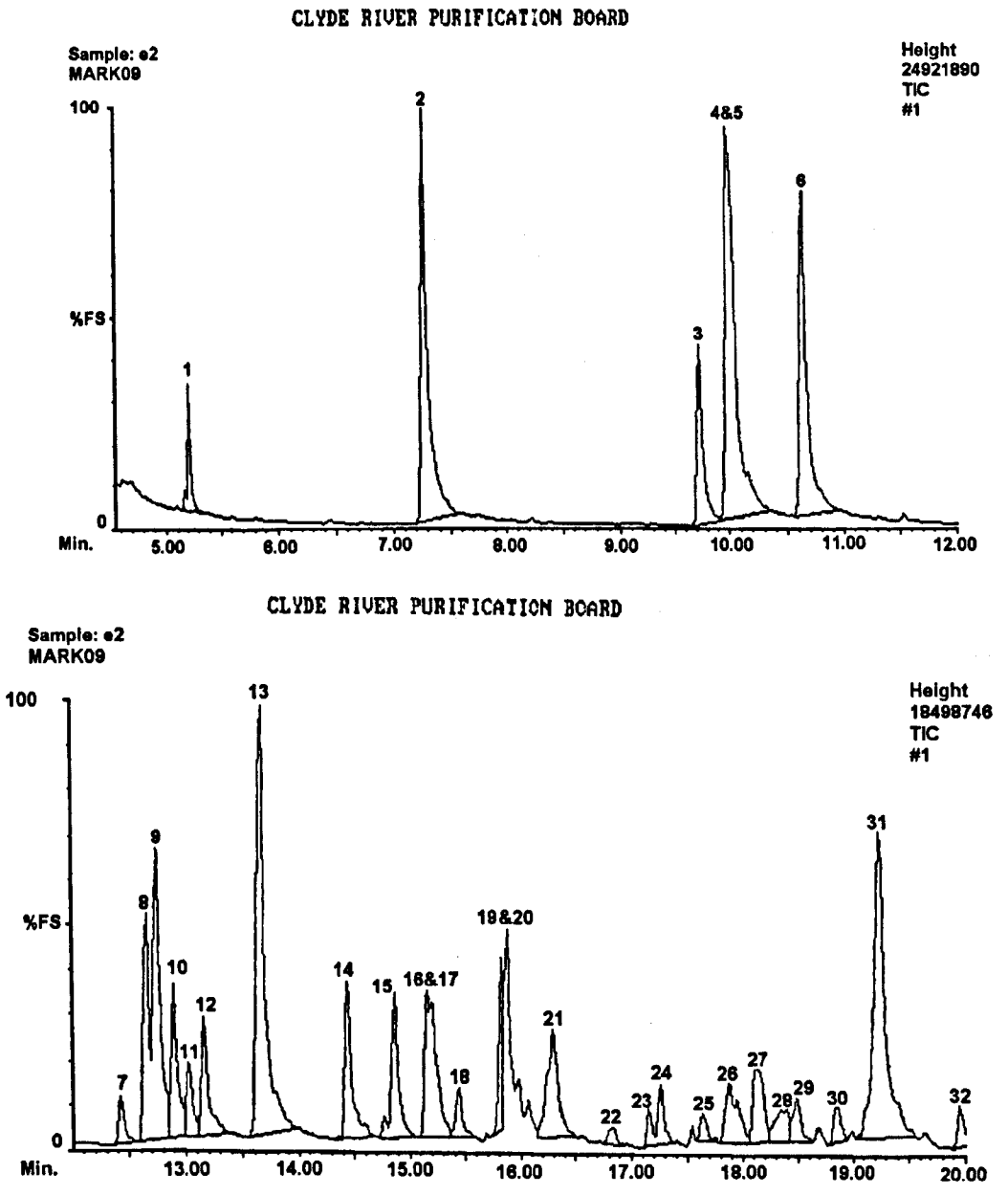

Fig. 3.3: Total Ion Chromatogram (TIC) of hydrocarbons in Outboard Motor Exhaust-Polluted Water (EPW), sampled. All peaks are numbered, corresponding to the scheme in Table 3.5.

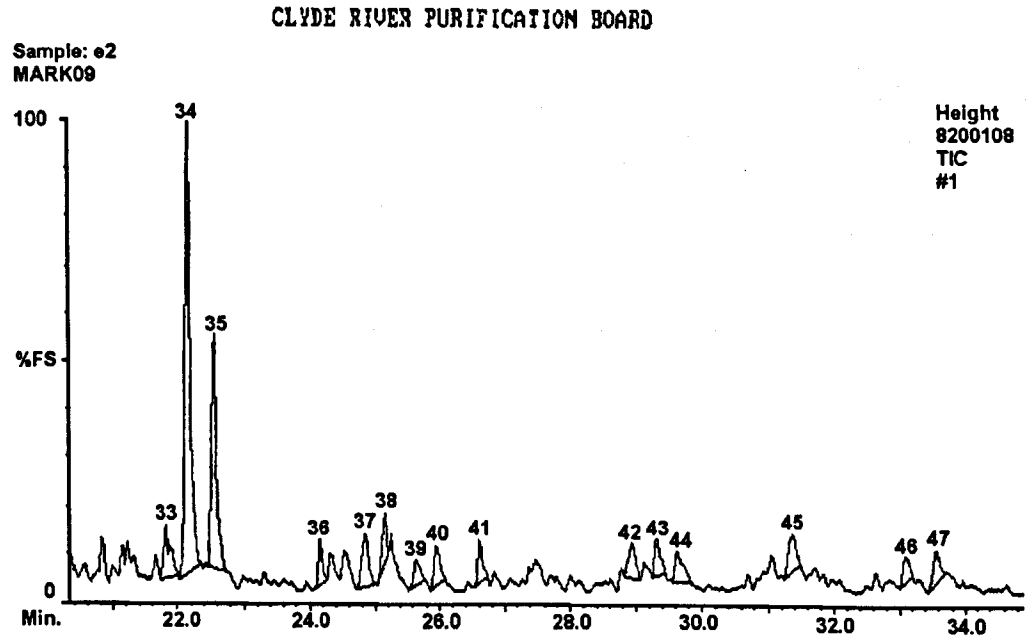

Fig. 3.3 (cont'd): Total Ion Chromatogram (TIC) of hydrocarbons in Outboard Motor Exhaust-Polluted Water (EPW), sampled. All peaks are numbered, corresponding to the scheme in Table 3.5.

Fig. 3.3 shows a chromatogram of the organic compounds in exhaust-polluted water taken immediately after running the engine in the tank. Forty-seven distinct peaks were detected, each corresponding to a different compound (numbered, corresponding Column 3 in Table 3.5). Table 3.5 lists these compounds, with detector peak heights shown.

Table 3.5: Organic compounds extracted from EPW with detector peak heights shown (in millions of ion counts). Compounds with confirmed identity (i.e. matching a standard) are **underlined in bold** and tentatively identified compounds (i.e. isomers of standards) are underlined in plain text. Approximately identified compounds (to nearest isomer) are named in plain text. Some compounds can only be identified to the nearest chemical group and these are indicated in *italics*, as are compounds of unknown identity.

Compound	Retention Time	Peak Number	Peak Height
Benzene	5.20	1	7.60
Methyl benzene (toluene)	7.27	2	24.49
Ethyl benzene	9.72	3	10.67
1,4-dimethyl-benzene	9.99	4	23.21
1,3-dimethyl-benzene	10.02	5	16.73
1,2 dimethyl-benzene	10.64	6	19.93
Propel-benzene	12.42	7	1.95
1-ethyl- 3-methyl benzene	12.62	8	9.30
1-ethyl- 4-methyl benzene	12.72	9	12.05
1,3,5-Trimethyl-benzene	12.89	10	6.32
Unknown	13.02	11	3.09
1-ethyl- 2-methyl benzene	13.15	12	4.81
1,2,4-Trimethyl-benzene	13.64	13	17.84
1,2,3-Trimethyl-benzene	14.44	14	6.58
Methyl ethenyl benzene	14.85	15	5.89
Methyl ethenyl benzene	15.15	16	6.36
2- or 3-methyl phenol	15.20	17	5.50
Diethyl benzene	15.44	18	2.01
4-methyl phenol	15.82	19	7.00
Methyl benzaldehyde	15.87	20	8.62
Methyl benzaldehyde	16.29	21	4.56
4C benzene	16.84	22	0.72
1,2,4,5-tetramethyl-benzene	17.15	23	1.49
1,2,3,5-tetramethyl-benzene	17.25	24	2.54
Unknown	17.64	25	1.11
Indene or unsat. 4C	17.87	26	2.41
Indene or unsat. 4C	18.10	27	3.01
5C benzene	18.34	28	1.39

Table 3.5 (cont'd): Organic compounds extracted from EPW with detector peak heights shown (in millions of ion counts). Compounds with confirmed identity (i.e. matching a standard) are **underlined in bold** and tentatively identified compounds (i.e. isomers of standards) are underlined in plain text. Approximately identified compounds (to nearest isomer) are named in plain text. Some compounds can only be identified to the nearest chemical group and these are indicated in *italics*, as are compounds of unknown identity.

Compound	Retention Time	Peak Number	Peak Height
2C benzaldehyde	18.49	29	1.90
Unknown	18.85	30	1.45
Naphthalene	19.22	31	12.59
Unknown	19.96	32	1.70
5C unsaturated benzene	21.82	33	0.88
2-Methyl naphthalene	22.14	34	7.71
1-Methyl naphthalene	22.54	35	3.99
Biphenyl	24.16	36	0.69
Dimethyl Naphthalene	24.84	37	0.88
Dimethyl Naphthalene	25.14	38	0.89
Dimethyl Naphthalene	25.64	39	0.44
Dimethyl Naphthalene	25.94	40	0.70
Methyl Biphenyl	26.59	41	0.76
Dimethyl Biphenyl	28.92	42	0.60
Diphenyl Propane	29.31	43	0.68
Diphenyl Propane	29.63	44	0.51
Unknown	31.36	45	0.60
Anthracene/ Phenanthrene	33.09	46	0.46
Unknown	33.56	47	0.47

3.3.1.2: Accuracy of Identification of Compounds in EPW

Table 3.6: Numbers of precisely identified compounds, tentatively identified compounds, approximately identified compounds, very approximately identified compounds and unknown compounds detected in the EPW sample analysed in the current study. The identification status of each compound is explained in Section 3.2.1.5 and the compounds themselves are listed in Table 3.5.

Identification status	Number of compounds
Precisely identified compounds	6
Tentatively identified compounds	14
Approximately identified compounds	15
Very approximately identified compounds	6
Unknown compounds	6

Table 3.6 shows the numbers of compounds detected in EPW, of each degree of precision of identification, as indicated in Table 3.2. Nearly all of the organic compounds detected were aromatic hydrocarbons from unburned fuel, with some phenols and benzaldehyde compounds present. Identifiable aliphatic compounds were found in trace quantities, by the use of a Partial Ion Chromatogram (PIC) of the ion with mass charge ratio (m/z) of 57. This PIC is shown in Appendix IV, with the identified compounds listed.

All six of the aromatic HCs, used as calibration standards (listed in Table 3.3) were precisely identified in the EPW sample. A further 14 compounds were tentatively identified by using library spectra combined with known elution sequences, from a previous study (Wang *et al.*, 1994). 15 compounds could only be approximately identified (to the nearest isomer) and six compounds could only be very approximately identified (to the nearest chemical group), using library spectra alone. The remaining six compounds (indicated by the remaining six peaks on the TIC) could not be identified: their library spectra were unreliable and contradictory, possibly due to their low concentrations.

3.3.2: Analysis of Fuel Hydrocarbons

Table 3.7 shows the compounds detected by GC-MS and accuracy of identification for each compound in the samples of outboard motor fuel:oil mixture, used in the current study. Chromatograms of a sample of the fuel:oil mixture used in the current study are displayed and interpreted in Appendix III. Table 3.8 lists the numbers of compounds of each degree of precision of identification (the same procedures used in Table 3.6 for the EPW sample). As can be seen, fewer compounds were detected than in the EPW sample, but precision of identification was higher.

Table 3.7: Compounds found in the uncombusted outboard motor fuel:oil mixture (0.1 % or 1000 ppm, w/v in DCM) analysed in this study. Compounds of confirmed identity are **underlined in bold** while tentatively identified compounds are underlined in plain text. Compounds identified only to the nearest isomer are indicated in plain text, while some compounds could only be identified to the nearest chemical group and these are indicated in *italics*. † = aliphatic compounds.

Benzene	**1,3,5-trimethyl-benzene**
3-methyl hexane †	1 ethyl- 2 methyl benzene
2,2,4-trimethyl-pentane†	1,2,4-trimethyl-benzene
Methyl benzene (Toluene)	1,2,3-trimethyl-benzene
n-octane †	*Unsaturated 3C benzene*
Ethyl benzene	*4C benzene*
1,4-dimethyl benzene	*4C benzene*
1,3-dimethyl benzene	*4C benzene*
1,2-dimethyl benzene	**Naphthalene**
Propyl benzene	2-Methyl naphthalene
1-ethyl- 3-methyl benzene	

Table 3.8: Numbers of precisely identified compounds, tentatively identified compounds, approximately identified compounds and unknown compounds detected in the fuel:oil mixture sample analysed in the current study (listed in Table 3.7).

Identification status	Number of compounds
Precisely identified compounds	5
Tentatively identified compounds	9
Approximately identified compounds	3
Very approximately identified compounds	4
Unknown compounds	0

3.3.3: Volatile Aromatic HCs in Loch Lomond

In Loch Lomond, volatile aromatic HCs were undetected in the surface waters of all three stations sampled in January 1994. On that day, no moving powerboats were observed in the whole south basin of the loch. By contrast, surface water samples taken in summer at all three stations clearly possessed volatile aromatic HCs. TICs of surface water samples from Balmaha Pier and the North Narrows (Site A and Site C, respectively - see Fig 3.2) in Loch Lomond are displayed and interpreted in Appendix V. The compounds detected are listed in Table 3.9. As in the case of the EPW, methyl benzene (toluene) is likely to be the most abundant compound (based on detector peak height), but other alkylated benzenes are also present. Some aliphatic compounds were also detected, including alcohols and cycloalkanes.

Table 3.9: Compounds detected at three sites in Loch Lomond during peak powerboating activity, with presence indicated by an asterisk.

Station →	Narrows	Balmaha	South Basin
Compound ↓			
Benzene	*	*	*
Methyl benzene (toluene)	*	*	*
Ethyl benzene	*	*	
1,4 dimethyl benzene	*	*	*
1,3 dimethyl benzene	*		
1,2 dimethyl benzene	*	*	
1 ethyl 3 methyl benzene	*	*	
1,3,5 trimethyl-benzene	*		
1 ethyl 2 methyl benzene	*		
1,2,4 trimethyl-benzene	*	*	
1,2,3 trimethyl-benzene	*		
Naphthalene	*		

At Site A (Balmaha Pier), fewer HC compounds were detected than at Site C. However, quite different substances, possibly nitrogenous aromatic compounds were also found (see Appendix V). These compounds were also detected in the North Narrows and South Basin sites, but in much lower concentrations (based on peak height). At Site B (mid-South Basin) only three HC compounds were detected.

3.3.4: Results: Basic Physical and Chemical Parameters

In the experimental tank, a statistically significant increase in bicarbonate concentration took place over the duration of the monitoring period each time the engine was run (see Fig. 3.4). Using Analysis of Covariance (ANCOVA) on $\log_{10}$ transformed data, it was found that there was no significant difference between the slopes of the individual regression lines (representing each experiment) ($F_{3,20} = 1.43$, n.s.), indicating

that the increase in bicarbonate concentration happened at a similar rate in each of the four experiments. Although there was a highly significant difference between the elevations of the regression lines ($F_{3,23} = 7.67$, $p < 0.001$) this was probably due to the differences in initial HCO_3 concentrations before each experiment. For the purposes of this analysis, the same number of data points from each experiment needed to be analysed. There were only eight common data points in all four of the experiments (see Table 3.4). In addition to this, the time (measured in days) was $\log_{10}$ transformed prior to analysis.

Data from each experiment were also examined individually, by regression analysis and the results are displayed in Table 3.10 and Fig. 3.4. The data confirm that significant increases in bicarbonate alkalinity took place in all four experiments. For the purposes of this regression analysis, all of the data collected (up to 12 days after commencement of each experiment) were used, unlike in the preceding ANCOVA. The only exception to this was the omission of the single sample taken after 43 days following the April 1994 experiment. The time (measured in days) was again $\log_{10}$ transformed.

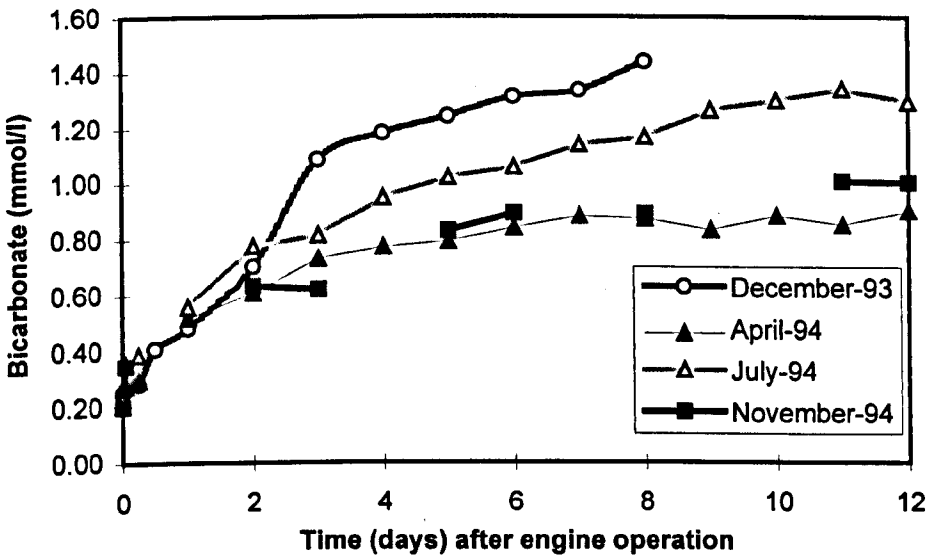

Fig. 3.4: Trends in bicarbonate levels in the outboard motor experimental tank in four experiments.

Table 3.10: Regression analysis of trends in bicarbonate levels in the outboard motor test tank: results of four engine trials.

Time of engine trial	r^2	t	d.f.	p
Dec. 1993	81.6 %	7.06	11	<0.001
Apr. 1994	94.2 %	15.15	14	<0.001
Jul. 1994	99.0 %	36.44	14	<0.001
Nov. 1994	97.1 %	16.26	8	<0.001

There was a chance that bicarbonate ions could have leached into the water from the aggregate material used to construct the experimental tank. In order to test this hypothesis, three control experiments were carried out, each immediately preceding and corresponding to the aforementioned engine trials of December 1993, April 1994 and July 1994. A statistically significant increase did take place in these control experiments ($t = 5.95$, 2 d.f., $p = 0.014$). However, when these increases were compared with the corresponding increases in the engine trials, the latter increases were found to be over three times greater and this difference (in the increase) was found to be highly significant ($t = 11.51$, 2 d.f., $p = 0.0007$), showing that most of the increases in bicarbonate concentration in these experiments occurred as a consequence of engine operation.

There were also statistically significant changes in pH ($t = 3.19$, $p = 0.033$, d.f. = 4) and temperature ($t = 2.96$, $p = 0.032$, d.f. = 5) in the first hour after the conclusion of engine operation, but no trends after this. Fig. 3.5 shows these changes in pH and temperature respectively, in each of the four engine trials. Oxygen levels showed no statistically significant trends at any time during engine operation in any of the four experiments.

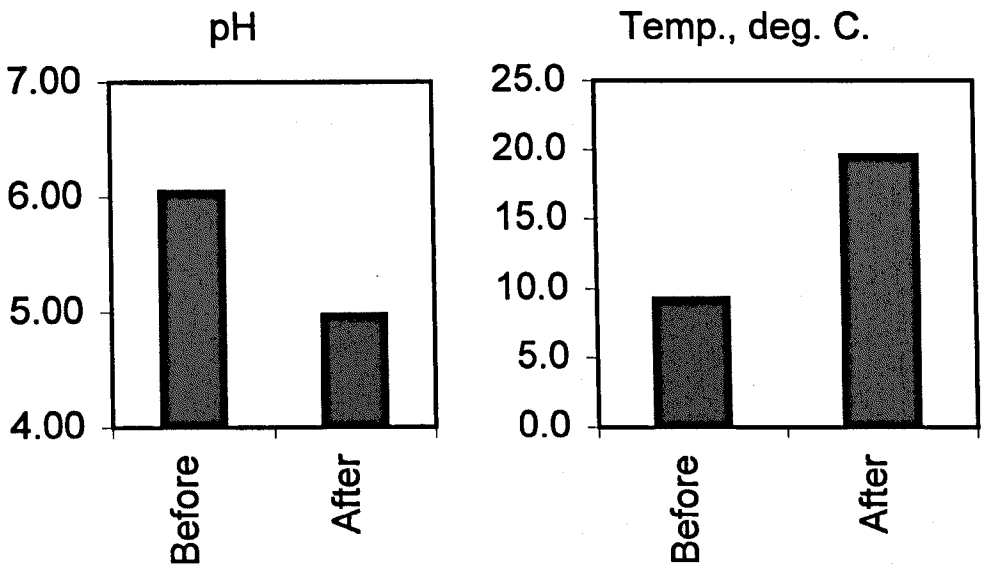

Fig. 3.5: Changes in pH and water temperature caused by running an outboard motor in the experimental tank. Mean of four analyses, before and after running the engine.

From samples collected during summer and winter on Loch Lomond, no differences in any of the physical or chemical parameters could be attributed to powerboat operation. Table 3.11 shows the results of the three most important sampling sessions, with surface water taken from five sites on Loch Lomond (see Fig. 3.2). On January 20 and August 7, water samples for hydrocarbon analysis were taken. On August 7, which was on a Sunday, boat densities were at their maximum for 1994 with 563 boats counted on the whole of Loch Lomond. Samples were also taken on a summer weekday (July 7) for comparison with the summer weekend and winter samples. Boat counts were not performed on January 20 and July 7, but from the results of a large scale boat survey carried out by Adams *et al.* (1992), boat densities were very likely to be zero on January 20. The numbers of boats on July 7 can only be estimated from summer weekday surveys, carried by Adams *et al.* (1992), in which the mean weekday boat number, for the whole of Loch Lomond in July 1989 (mean of 5 censuses) was 145. This is only 26% of the number of boats counted on August 7.

Table 3.11: Basic physical and chemical parameters of the surface waters of Loch Lomond, at five sites and on three occasions, representative of a winter weekend (20.1.1994), a summer weekend (7.8.1994) and a summer weekday (7.7.1994). Oxygen is measured in percentage saturation and bicarbonate (HCO_3^-) levels are measured in millimoles per litre.

Date	Station	T°C	Oxygen	pH	HCO_3^-
20.1.1994	A	3.0	92	6.11	0.490
	B	3.5	94	5.99	0.227
	C	4.0	94	5.80	0.202
	D	3.5	96	6.06	0.248
	E	4.5	94	5.78	0.186
7.7.1994	A	15.5	100	6.13	0.323
	B	15.0	98	6.46	0.225
	C	15.0	96	6.11	0.216
	D	15.0	98	6.12	0.265
	E	15.0	96	6.44	0.217
7.8.1994	A	18.0	117	6.29	0.331
	B	18.0	120	6.25	0.233
	C	18.0	118	6.11	0.205
	D	17.5	120	6.22	0.291
	E	18.0	119	6.32	0.220

3.4: Discussion

A number of important objectives were achieved in the experiments described in the current chapter, including:

1) Appropriately contaminated water was successfully produced and the chemical characterisation of this contaminated water with respect to HCs was carried out (see Section 3.4.1).
2) The identification of 20 of the characteristic organic compounds in EPW (see Section 3.4.1).
3) The detailed analysis of the aromatic HCs in uncombusted outboard motor fuel:oil mixture (see Section 3.4.2).
4) The detection of volatile aromatic HCs in the surface waters of three sites in Loch Lomond (see Section 3.4.3).
5) The effect of powerboat operation on some basic physical and chemical parameters was investigated, both in controlled and field experiments (see Section 3.4.5).

3.4.1: Hydrocarbons In Exhaust-Polluted Water (EPW)

The results of the analysis of EPW, in the current study agree well with the only two previous comparable studies, especially that of Jüttner *et al.* (1995*a*). In that study, a remarkably similar range of organic compounds in EPW were found, including a few phenolic and benzaldehyde compounds. The latter were probably products of partial combustion of the fuel. In the study by Montz *et al.* (1982), less accurate analytical equipment was used but this was compensated by quantifying compounds precisely, by carrying out seven engine operation trials and analysing samples from each, by GC-FID (Gas Chromatography, with a Flame Ionisation Detector). Again, very similar compounds were detected to those in the present study.

One notable feature of one of these previous studies is the far higher emission rate of two-stroke outboard motors compared to four-strokes (Jüttner, 1994; Jüttner *et al.*, 1995*a*). Using very similar experimental conditions for the analysis of contaminated water from both types of engine, it was found that two-stroke engines emitted over fifty times as much HC material to the water. This is a greater difference than that found by Mele (1993), in which two-stroke engines were found to be fourteen times as polluting.

Although it was found that a large proportion of the volatile HCs emitted during engine operation may not remain in the water for any significant time period (Wachs *et al.*, 1982; Miller and Fiore, 1997), those compounds which do are known to be toxic. In a wide-ranging study on marine oil pollution (NRC, 1985), toxicity data of several different hydrocarbon compounds to a diverse range marine organisms was tabulated. All of these compounds had relatively high toxicity (see Kuhn *et al.*, 1989*b*), particularly Polycyclic Aromatic Hydrocarbons (PAHs), which have been found in petrol and lubricant oils and in trace quantities in EPW in the present study (See Table 3.5). PAHs are known carcinogens (McKee and Plutnick, 1989; Cavalieri *et al.*, 1991) and have the highest toxicity of any of the HC compounds in petroleum. They also have the potential to bioaccumulate, both in freshwater organisms (Southworth *et al.*, 1978; van der Oost *et al.*, 1994) and marine organisms (Mc.Donald *et al.*, 1992; Hellou *et al.*, 1993, 1994; Shchekaturina *et al.* 1995).

3.4.2: Fuel:Oil mixture

A very similar range of aromatic HC compounds were found in fuel compared to EPW. This indicates that the vast majority of organic compounds entering water from powerboat exhausts are unburned fuel HCs (principally volatile aromatic HCs, such as benzene and alkylated benzenes).

The fuel:oil mixture sample was probably much more dilute than the extract of EPW. Therefore only the major components (21 compounds) in the fuel:oil mixture (which has hundreds more HC compounds – see Johansen *et al.*, 1983) were detected. Since these major compounds were easy to identify, most of the compounds in the fuel:oil mixture were reasonably accurately identified compared to those in EPW. In the EPW sample, more compounds were detected, due to the probable greater overall concentration of the sample. This allowed minor components to be present in the sample at detectable levels.

3.4.3: Analysis of Powerboat Exhaust Pollutants in Loch Lomond

The types of aromatic HCs in the samples from Loch Lomond suggest that their source is unburned fuel, either from spillage or emissions. The former is possible at site A, which is an area of relatively intensive boat servicing, and the latter is more likely at site C (see Fig 3.2), which is situated in boat census survey Area 5, which is subject to some of the highest powerboat activity in Loch Lomond (see Adams *et al.*, 1992). Similar compounds were found in freshwater (Miller and Fiore, 1997) and marine ecosystems (Mantoura *et al.*, 1982), both studies making the same deduction as the current study, namely that powerboat exhaust was responsible for water pollution by petroleum HCs. Methyl-tert-butyl-ether (MTBE), detected in Lake Tahoe by Miller and Fiore, was not found in any of the samples in the present study. Previous research (Johansen, 1983) has shown that MTBE is not usually added to leaded petrol, which was used in the experiments described in this chapter.

There are other potential sources of HC pollution in Loch Lomond, namely atmospheric fallout and road runoff. Atmospheric fallout occurs when precipitation washes airborne chemicals, either in particulate form or adsorbed onto particles, out of the atmosphere and into aquatic ecosystems. Previous studies have shown that atmospheric deposition is a major source of PAH and lead in aquatic ecosystems (Sanders

et al., 1993; Pham *et al.*, 1993). However, no HCs of any type were detected at any of the three sites in Loch Lomond in winter, when rainfall is highest, despite the fact that most atmospheric deposition of organic compounds in freshwaters occurs from rainfall (Harrison and Peak, 1995). This suggests that significant input of volatile aromatic HCs from atmospheric fallout is highly unlikely to occur.

The other interfering source of hydrocarbon pollution is road runoff (Marsalek and Schroeter, 1988; Evans *et al.*, 1990; Murphy *et al.*, 1993). This could be investigated in future work, by analysing samples of road runoff-contaminated water. As in the case of atmospheric fallout, contamination by road runoff is unlikely to have been significant compared to pollution from powerboat exhausts in Loch Lomond. Road runoff pollutants consist mostly of aliphatic HCs and PAHs (Bomboi and Hernandez, 1991; Boxall and Maltby, 1995). Volatile aromatic compounds from road vehicles are not significant pollutants in road runoff and are mostly emitted to the air (Jüttner *et al.*, 1995*a*) and would not be expected to reach water by any resultant atmospheric deposition or directly by road runoff.

One unexpected observation was the presence of relatively high quantities of organic compounds which were very different to petroleum HCs. These substances may have been nitrogenous aromatic compounds and although the exact origin of these compounds is unknown, a possible source is from boat cleaning operations in this area (Pirie, *pers. comm.*).

3.4.4: Limitations of HC Analysis by GC-MS in the Current Study

3.4.4.1: Spillage from the Tanks in Controlled Experiments

One significant source of inaccuracy in the controlled experiments was spillage of water from the tank during engine operation. In the controlled experiment, 168 litres or 32.9 % of the tank's volume was lost during engine operation. This made it difficult to estimate the amount of water that was contaminated in the experiment. This was assumed to be the final volume of water in the tank after engine operation, as it was observed that nearly all spillage occurred during the first two minutes (out of 1 hour) of engine operation. In the experiments investigating the effects of outboard motor operation on basic physical and chemical parameters (see section 3.2.5 in this chapter) using the same tank, an even greater amount of spillage occurred. In one experiment, over half of the tank's volume was lost. Some water even needed to be added to the tank during engine operation to prevent the water level dropping too low.

3.4.4.2: Limitations of the GC-MS Analytical Procedure Itself

The computer programme used in the GC-MS analysis in the current study very precisely detected aromatic compounds, as ionic fragments from 50-500 a.m.u. were detected. Appendix II describes mass spectra of aromatic and aliphatic compounds detected in samples in the current study. Aromatic compounds usually shatter into fragments of more than 50 a.m.u.'s (McLafferty, 1980) while, as mentioned in Section 3.4.2, many of the aliphatics in the fuel sample were almost undetectable and not quantifiable, as a large proportion of their fragments are of less than 50 a.m.u. in mass and were not detected or quantified in this study. A more wide ranging MS programme, detecting smaller ionic fragments which comprise a large proportion of the ionic fragments of aliphatic compounds, especially volatiles, could have been useful for more thorough analysis of fuel samples. This would also help to see which fuel compounds

evaporate or degrade most quickly.

In contrast to the aromatics normally found in petroleum, the corresponding aliphatics have a much greater number of different isomers. Although the total amount of these compounds present is considerable, most of them may have been present in concentrations too low to be easily detected by the GC-MS apparatus in the current study. In addition to the MS operating conditions, the column used was most appropriate for the analysis of compounds of medium polarity, such as aromatic HCs. Aliphatic HCs have relatively low polarity (Loudoun, 1988).

Despite the limitations in detecting aliphatic compounds in the present study, it is highly unlikely that any significant quantities of these compounds enter the water as a result of powerboat operation. Jüttner (1994) analysed ionic fragments between 28.5 and 350 a.m.u. and still did not detect any aliphatics in EPW. Such a programme would have easily detected them if they occurred. In addition, Montz *et al.* (1982) only detected traces of aliphatics in EPW.

3.4.5: Discussion: Basic Physical and Chemical Parameters

Some basic physical and chemical parameters (bicarbonate levels, temperature and oxygen saturation) were effected by the operation of the outboard motor tank, due to the very small volume of water present, and the absence of any other major influencing factors. There may have been some leaching of bicarbonate from the aggregate material of the tank, but further experiments showed that this was not likely to be very significant when compared to the increase that occurred as a result of engine operation.

After engine operation, bicarbonate levels were still elevated after 12 days, and in order to briefly investigate longer term trends, one sample was taken 43 days after the commencement of the trial, beginning April 12th. Bicarbonate levels were found to

remain high (1.4 millimoles per litre).

In the case of Loch Lomond, water temperatures were typical for the times of the year at which samples were taken, with no exceptional readings. This was also the case with pH measurements, which displayed very little variation. Oxygen levels were high on some occasions, including August 7, but supersaturation can often naturally occur in the surface waters of Loch Lomond (Habib *et al.*, 1997). Only one elevated bicarbonate measurement occurred within the three most important surveys, and this occurred in winter, when boating levels were negligible. Factors other than powerboat densities, such as weather conditions and time of year were most likely to be responsible for the variation in the physical and chemical parameters analysed in the present study. Although further water samples were taken from Loch Lomond in the current study (Ch.5), none of the aforementioned basic parameters were measured, as this was deemed to be superfluous, based on the findings described here.

3.4.6: Further Research

3.4.6.1: Further Research in the Current Study

Since volatile aromatic HCs were found to be detectable at some locations in Loch Lomond, it was then necessary to find out the distribution of these compounds throughout the loch. Further experiments, described in Chapter 5, were carried out in order to examine the temporal and spatial (including vertical) distribution of volatile aromatic HCs in Loch Lomond. In order to achieve this, a large number of water samples needed to be taken and a new method was developed to efficiently identify and quantify volatile aromatic HCs in water. Chapter 4 describes the development and capabilities of this method. A final problem posed by the presence of volatile aromatic HCs in Loch Lomond is the potential for these compounds to cause ecological damage. To gain an

indication of this, toxicity tests using these compounds and a brief environmental risk assessment of them were carried out. Chapter 6 describes these investigations.

3.4.6.2: Other Further Research

In the current study, traces of Polycyclic Aromatic Hydrocarbons (PAHs) were detected in outboard motor Exhaust-Polluted Water (EPW). Previous work has demonstrated that PAHs are very toxic (NRC, 1985; McKee and Plutnick, 1989) and can accumulate in sediment (Wade *et al.*, 1994; Huntley *et al.*, 1995). In one previous study (Mastran *et al.*, 1994), PAH levels in the water and sediment of a drinking water reservoir were measured. Powerboating activity was found to be a source of PAH contamination. Similar future work could be useful in the case of Loch Lomond, in order to evaluate PAH input from powerboats to both water and sediment, especially in areas of locally high powerboat density.

Previous work has shown that PAHs can bioaccumulate in zooplankton. Southworth *et al.* (1978) demonstrated the bioaccumulation of several PAH compounds in *Daphnia pulex* and found that accumulation was greater for heavier compounds with more benzene rings. Some other compounds may increase in concentration with increasing trophic level, a phenomenon known as biomagnification (Phillips, 1993), which has been demonstrated for some types of toxic pollutants, such as Poly Chlorinated Biphenyls (PCBs: see Porte and Albaieges, 1994). PAHs do not show any evidence of biomagnification (Broman *et al.*, 1990), as they are partially metabolised in fish and other vertebrates higher up the food chain than Daphnids. The intermediate metabolites produced may indicate harmful exposure to PAHs and these intermediates, found in the bile of fish, may be at least as toxic as the parent PAH compounds (McDonald *et al.*, 1992; van der Oost *et al.*, 1994). Another indicator of PAH contamination is the formation of DNA adducts, also in the liver of fish. In the case of Loch Lomond, PAH levels in zooplankton and in the bile of planktivorous fish (such as powan, *Coregonus*

lavaretus) and the presence of DNA adducts could be monitored, in order to assess the risk of PAH toxicity to organisms at different trophic levels.

Chapter 4: The Development of a Modified Method for the Analysis of Volatile Aromatic Hydrocarbons in Freshwaters

4.1: Introduction

4.1.1: Previous Research in Hydrocarbon (HC) Pollution

In recent years, there has been a substantial quantity of literature produced on the subject of marine oil pollution (NRC, 1985; Pople *et al.*, 1990; Sauer *et al.*, 1993; Turrell, 1994). By contrast, relatively few studies have examined HC pollution in freshwater ecosystems. Marine (usually crude) oil spills are usually acute, large-scale pollution incidents and produce easily observable effects. Consequently, a lot of publicity is generated with a resulting high level of public awareness, which is lacking in incidents of freshwater HC pollution (which generally tend to have less easily observable effects). Despite this, some types freshwater HC pollution have been studied, notably:

- Road runoff (Boxall and Maltby, 1995; Bomboi and Hernandez, 1991)
- Groundwater contamination (Maher *et al.*, 1990)
- Occasional crude oil spills (Poulton *et al.*, 1997)
- Pollution by powerboat engines (Montz *et al.*, 1982; Jüttner *et al.*, 1995*a,b*)

A wide variety of analytical techniques have been used in the detection and measurement of petroleum hydrocarbons (HCs) (mainly from crude oil spills) in the marine environment, e.g. gravimetric analysis (Sauer *et al.*, 1993), infra-red spectroscopy (Anderson *et al.*, 1980; ASTM, 1980*a*) and Gas Chromatography-Mass Spectrometry (GC-MS: see Page *et al.*, 1988). Another technique presently in widespread use is Fluorescence Spectroscopy (see Law *et al.*, 1988; MEWAM, 1988; John and Soutar, 1976, 1981). Fluorescence Spectroscopy has several advantages over most other techniques:

- It is relatively fast (unlike GC-MS)
- It is much less expensive than chromatographic analyses.
- It is very sensitive (more than any of the previously mentioned techniques, with the exception of GC-MS)
- It is non-destructive, allowing further analyses by other techniques.
- It can distinguish between different oils.

4.1.2: Context of the Current Study (in Ch. 4)

In this chapter, the development of a modified Fluorescence Spectroscopy method to detect and measure volatile aromatic HCs in freshwaters, is outlined. Previous work (Jüttner, 1994; Montz *et al.*, 1982), has shown that over 80% of the organic material entering the water column from powerboat exhausts consists of volatile monocyclic aromatic HCs from unburned fuel, i.e. benzene and its derivatives. Knowing the characteristic compounds of powerboat HC pollution, it was intended to perform a large-scale water quality survey of Loch Lomond, to investigate the temporal and spatial distribution of these compounds (Ch. 5). Such a survey was not practicable without the capability of analysing large numbers of water samples quickly, and the modified method aimed to achieve this capability.

HC analysis techniques in general are reviewed in Appendix I, in addition to the reasons for selecting the relevant techniques for the current study.

4.1.3: Aims

General Aim

- To develop a reliable, but simple analytical method, capable of detecting powerboat exhaust HC pollutants in freshwater.

Specific Aims

- To develop methodology which allows relatively large numbers of samples to be analysed rapidly.
- To be able to appropriately calibrate the detection apparatus so as to allow accurate measurements.
- To measure the extraction efficiency of powerboat exhaust HC compounds from water.
- To confirm the effectiveness of the modified method (which must be non-destructive) with a secondary analysis of a limited number of samples, using an established method.

4.2: Principle of the Method

4.2.1: General Principle of Fluorescence Spectroscopy in Hydrocarbon (HC) Analysis of Water Samples

All aromatic hydrocarbons (HCs) fluoresce. A significant proportion of almost all petroleum-derived oils (including petrol and diesel) is formed by aromatic HCs. As these compounds are the most soluble components of such oils, they comprise the majority of their water-soluble components (NRC, 1985). The presence of petroleum oils in water can therefore be confirmed from the presence of aromatic HCs, which can be detected by an appropriate method which measures the fluorescence of these compounds. There have been a number of studies in which such methods have been developed for the detection, identification and measurement of petroleum HCs from crude oil in water (John and Soutar, 1976, 1981; MEWAM, 1988; Law *et al.*, 1988).

4.2.2: General Description of the Procedure of Fluorescence Spectroscopy

When an appropriately prepared sample containing aromatic HCs is subjected to electromagnetic (EM) radiation in the ultra violet to visible range (UV-visible, from 200-500 nm wavelength), the energy gained by the aromatic HCs is partly given off as fluorescence, whereby EM radiation of a lower energy level (and consequently longer wavelength) is emitted (John and Soutar, 1981; Lakowicz, 1983). This emitted EM radiation can then be measured by a fluorimeter. For a given class of aromatic HCs (in the case of this study, monocyclic aromatic HCs), the amount of fluorescence emitted is proportional to the amount of aromatic HCs present. For a more detailed description of the principles of fluorescence spectroscopy, the reader is referred to Lakowicz (1983).

4.3: Procedure of Method Development

4.3.1: Analysis of Standard Solutions

4.3.1.1: Methods

Before any analysis, all glassware was very thoroughly washed, to remove contaminants, either HCs or any other fluorescing materials. The procedure is described in Ch. 3 (see section 3.2.1.1).

In order to confirm that the fluorimeter available (Spex Fluoromax dm 3000 f) could detect powerboat exhaust HC compounds, an initial step in method development required an analysis of standard solutions. The substances analysed were:

- Benzene
- Methyl benzene (toluene)
- 1,2 dimethyl benzene
- 1,3 dimethyl benzene
- 1,3,5 trimethyl benzene
- 50:1 petrol:oil mixture

These standards were diluted at concentrations of approximately 10 $mg.l^{-1}$ (using micro-pipettes) in both dichloromethane (DCM) and hexane. Hexane and DCM are standard extraction solvents, used in fluorescence analysis of water samples polluted by crude oil (see Gordon and Keizer, 1974; MEWAM, 1988; Siron *et al.*, 1991). Pure hexane and DCM were also analysed using the same conditions. Extracts of aqueous samples were not analysed at this stage, as the extraction process could have introduced unnecessary variation. The pure compounds listed above are all present in petrol (Ch. 3; Johansen *et al.*, 1983; Durand *et al.*, 1987) and in outboard motor exhaust-polluted water (EPW - see Ch. 3; Jüttner, 1994; Jüttner *et al.*, 1995a).

The 50:1 petrol: lubricant oil mixture is a standard fuel used to propel many outboard motors, which comprise the majority of powered recreational craft in both the UK (LDNPA, 1994), including Loch Lomond (Ch. 2) and the USA (Mele, 1993). Previous work in the current study (see Sections 3.3.1 and 3.3.2) has shown that the aromatic compounds in the 50:1 fuel:oil mixture are very similar to those entering water from powerboat exhaust both in the compounds present and their relative proportions. Therefore, the fuel:oil mixture is a good representative standard to develop techniques for testing for exhaust emissions.

Analysis of the aforementioned standards was by Synchronous Scanning Fluorescence Spectroscopy (SSFS), with an emission offset of 50 nm. This process involved subjecting the samples to EM radiation of all excitation wavelengths ranging from 220 to 450 nm. For each integral excitation wavelength, the emitted fluorescence at a wavelength of 50 nm greater than this was measured. All fluorescence measurements are expressed in counts per second (or cps). Previous research has shown that most petroleum oils will emit fluorescence at wavelengths of 270 to 500 nm (John and Soutar, 1981; MEWAM, 1988; Law *et al.*, 1988). SSFS was chosen for such preliminary analyses, as it gives the best indication of the general pattern of emitted fluorescence at different wavelengths. Such techniques are useful for distinguishing different oils or hydrocarbon mixtures by examining their fluorescence patterns or "fingerprints" (see John and Soutar, 1976; MEWAM, 1988).

An offset of 50 nm was chosen because previous studies have analysed crude oils by the closely related technique of emission scanning fluorescence spectroscopy. This involves subjecting a sample to EM radiation of a single wavelength and measuring the emitted fluorescence at a range of wavelengths. Using this method, it was found that the peak fluorescence of crude oil HCs was located at a wavelength 50 nm higher than the excitation wavelength. (MEWAM, 1988; Law *et al.*, 1988; Carls and Rice, 1988). It was therefore felt that a similar difference between the appropriate excitation and emission wavelengths was likely to occur in petrol, in the current study.

4.3.1.2: Results

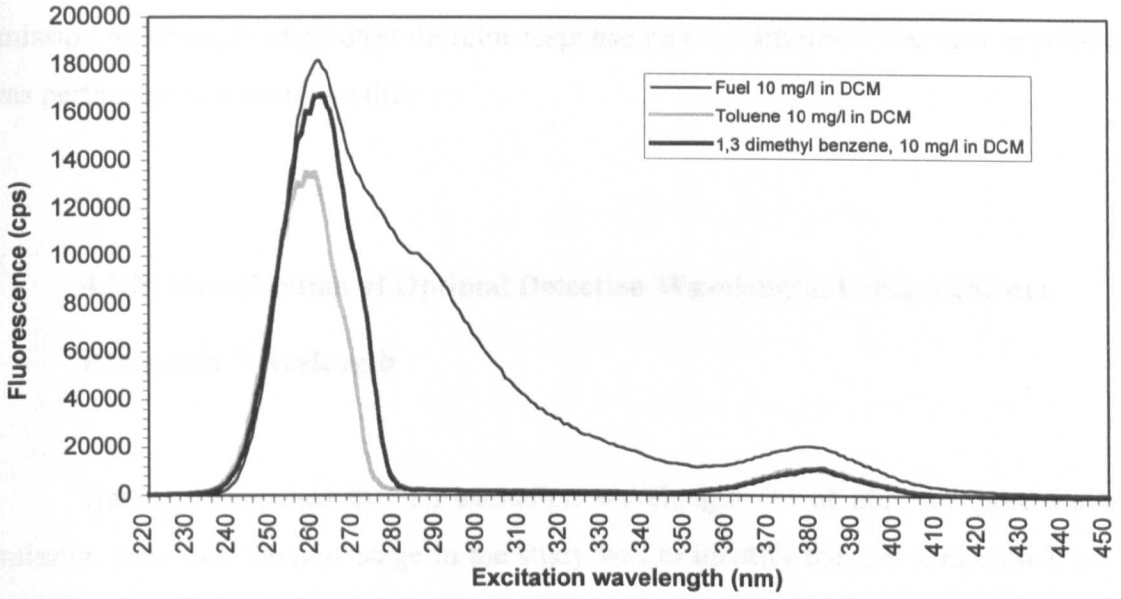

Fig. 4.1: *Results of SSFS of pure standards of fuel:oil mixture, toluene and 1,3 dimethyl-benzene, each substance diluted in pure DCM.*

Fig. 4.1 shows the SSFS scans produced by fuel:oil mixture, methyl-benzene (toluene) and 1,3 dimethyl-benzene (2 of the most abundant aromatic compounds known to be present in petrol - see Johansen *et al.*, 1983) in DCM. The pure compounds yielded very similar scans, with the highest emitted fluorescence measured at an excitation wavelength of 262 nm (corresponding to the peak in the graph), and very little emitted fluorescence at other excitation wavelengths. While the fuel:oil mixture yielded a slightly less well-defined response (with more fluorescence emitted at other excitation wavelengths), peak emitted fluorescence was also found at the excitation wavelength of 262 nm. The samples diluted in hexane yielded very similar fluorescence patterns, with the peak emitted fluorescence at the same wavelength, but the amount of fluorescence (or "quantum yield"; measured in cps) was only about two-thirds as much as that found in the DCM standards. This shows that DCM performed better than hexane as a solvent medium for volatile aromatic HC samples. This could be due to more absorption of EM radiation by hexane than by DCM .

These results show that the best fluorescence response from volatile (monocyclic) aromatic HCs is gained by using an excitation wavelength of 262 nm. The fluorescence gained at an emission wavelength of 312 nm (using a 50 nm offset) is known, but the emission wavelength of greatest detector response may be different. The next experiment was performed to investigate this.

4.3.2: Identification of Optimal Detection Wavelength, Using a 262 nm Excitation Wavelength

Having established that an excitation wavelength (λ) of 262 nm gave the best emission response, the next stage in the study was to identify the best emission λ for an excitation λ of 262 nm.

4.3.2.1: Methods

To find the optimum emission wavelength for the detection of volatile monocyclic aromatic HCs, the samples analysed by SSFS (section 4.3.1) were kept for a second analysis. This involved using only one excitation wavelength, 262 nm (which gave the optimum fluorescence response by SSFS), and measuring the emitted fluorescence at all wavelengths greater than this, up to 450 nm, thereby producing an emission scan.

4.3.2.2: Results

Fig. 4.2 shows emission scans of the same three standard samples (diluted in DCM), previously analysed by SSFS. As can be seen, the greatest emitted fluorescence is found at a wavelength of 286 nm in all three samples, indicating that volatile monocyclic

by SSFS manifested similar emission scans to those of toluene and 1,3 dimethyl-benzene. Fuel:oil mixture showed a less well defined response than that of the pure compounds, with more emitted fluorescence at wavelengths other than 286 nm.

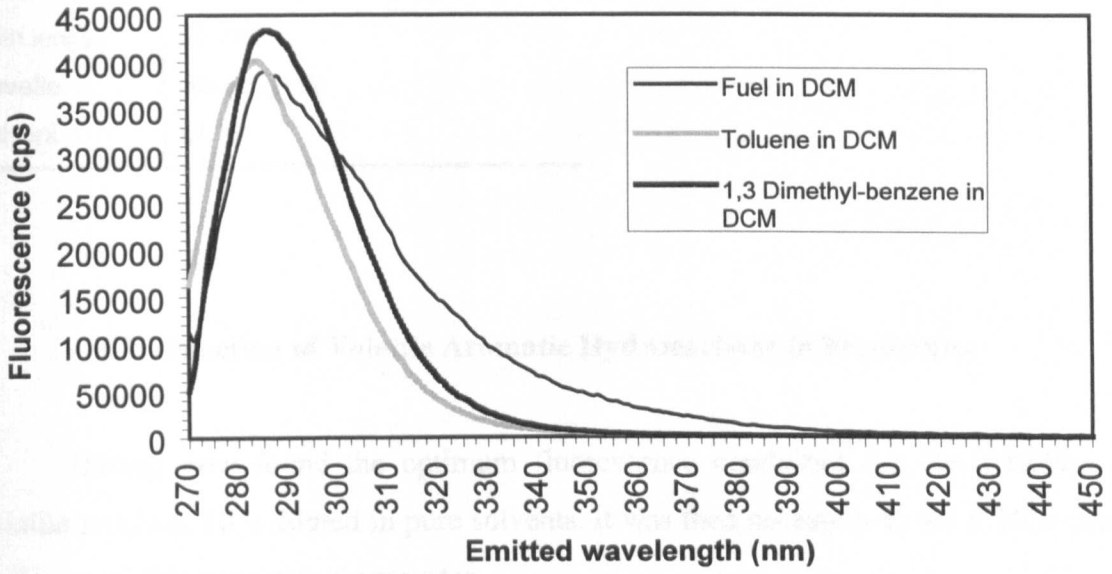

Fig. 4.2: *Emission scans (excitation λ of 262 nm) of pure standards of fuel:oil mixture, toluene and 1,3 dimethyl-benzene, each substance diluted in pure DCM.*

Thus far, the optimum fluorescence spectroscopy analytical conditions, determined in the present study for the measurement of volatile monocyclic aromatic hydrocarbons are displayed in Table 4.1.

Table 4.1: Fluorescence conditions used in the present study.

Fluorimeter	Spex Fluoromax dm3000f
λ excitation	262 nm
λ detection (emission)	286 nm
Sensitivity	1 mg.l-1
Path length	10 mm
Cuvette	Silica quartz
Solvent	DCM

4.3.3: Detection of Volatile Aromatic Hydrocarbons in Freshwater

Having now found the optimum fluorescence conditions for the detection of volatile aromatic HCs diluted in pure solvents, it was then necessary to see if HCs could be detected after extraction from water.

4.3.3.1: Methods

In order to test the ability of the fluorescence technique to detect volatile aromatic hydrocarbons (HCs) in freshwater, twenty water samples of 900 ml volume, were taken from Loch Lomond at a site in the Field Station Bay (Ordnance Survey Grid Reference NS 377 955). Of these samples, ten were extracted with DCM and the other ten with hexane in order to find out which solvent was most appropriate for this procedure. Ch. 3, section 3.2.1.3 describes the extraction procedure, which was identical for both solvents. There was one difference however, as the final sample volume was only reduced to 5 ml in this experiment (not 1 ml).

Following extraction, seven samples from each set were spiked with known concentrations of 50:1 fuel:oil mixture, to give total concentrations after extraction of 2, 5, 10, 20, 50, 100 and 500 $mg.l^{-1}$. The three other replicate samples consisted of:

1) Pure Loch Lomond water, also taken from the Field Station Bay area of Loch Lomond
2) A relatively lightly contaminated sample, produced by driving the sampling boat slowly forward while collecting water from behind the exhaust outlet. The engine on this boat was a "Mariner" 75 hp two-stroke outboard motor.
3) An obviously contaminated water sample, produced by running the aforementioned outboard motor at full throttle in neutral gear for 2 minutes at the Field Station jetty in Loch Lomond (Ordnance Survey Grid Reference NS 377 959) and then collecting the water from directly behind the engine's exhaust.

The replicate samples Nos. (2) and (3) were taken penultimately and last respectively, to avoid contamination of the other samples from engine exhaust pollutants.

Following extraction, all samples were analysed by emission scanning fluorescence spectroscopy, with an excitation wavelength of 262 nm and detection (emission) wavelength of 286 nm (see Table 4.1).

4.3.3.2: Results

In all samples, a large background fluorescence was present at all emission wavelengths scanned. This interference manifested itself as a yellow colour and was most likely to be caused by the presence of humic acids, lipids and other biogenic materials present in freshwaters and extractable with DCM and hexane. Despite this interference, aromatic hydrocarbons (HCs) in most of the spiked samples and all of the exhaust samples were clearly observable. In the case of the former, only concentrations of fuel:oil mixture (in the extracts in DCM) of 5 $mg.l^{-1}$ or less did not cause detectable increases in fluorescence (see Fig. 4.3). In all other samples (spiked and exhaust), aromatic HCs are

indicated by clearly elevated fluorescence at emission wavelengths of 286 nm and 307 nm.

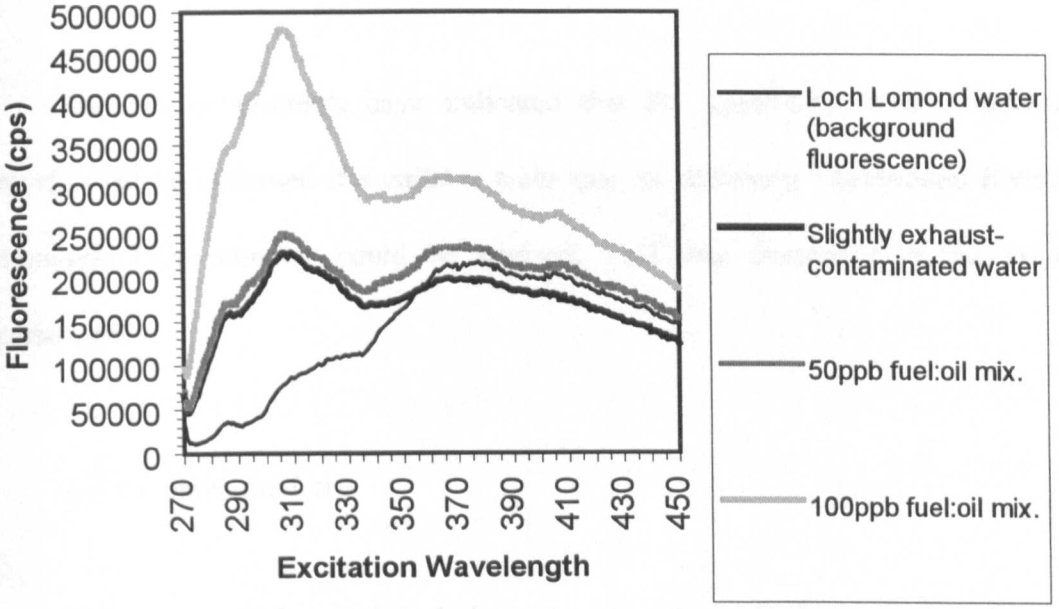

Fig. 4.3: *Emission scans of the first water samples from Loch Lomond (excitation wavelength 262 nm). Lightly exhaust-contaminated water is compared to two spiked water samples of low hydrocarbon (HC) concentration.*

However, further sampling from Loch Lomond showed that the background interference varied greatly, both temporally and spatially. Such a variable interference needed to be removed for any subsequent meaningful studies. Hexane was ruled out as an extraction solvent at this stage, as in addition to the sensitivity being considerably lower than that obtained using DCM, the interfering background fluorescence was relatively much greater. This suggests that hexane was much more efficient at extracting compounds from water associated with background fluorescence, while being less efficient at extracting volatile aromatic HCs.

4.3.4: Removal of Background Fluorescence from Compounds that Prevent Clear Detection of HCs in Water

Previous experiments have indicated that the sensitivity of the fluorescence method could be increased if a suitable technique for removing background fluorescing compounds from samples could be devised. This was attempted in the following experiments.

4.3.4.1: Introduction

The most widely used technique for the removal of interfering fluorescing materials is normal phase column chromatography, whereby samples held in solvents of relatively low polarity (such as DCM; the moving phase) are passed through a column packed with an appropriate adsorbent (the stationary phase). Polar impurities (such as humic acids and lipids) are trapped in the column by the adsorbent, while the less polar determinands (in this case, volatile aromatic HCs) pass through the column and are eluted with DCM (see Snyder 1968, for a detailed description of column chromatography). In the current study, a combination of different sample volumes and stationary phase types and volumes were investigated for their efficacy.

4.3.4.2: Methods

(a): Preparation of Materials

Prior to these investigations, all glass columns (all of them 9.6 mm in internal diameter) were thoroughly cleaned by rinsing four times in tap water and distilled water followed by oven drying at 100°C for 1 hour, then cooling and finally washing 4 times in DCM.

The specifications of the two gels tested were:

i. Aluminium oxide (Al_2O_3) 90, particle size 0.063 to 0.200 mm (70 – 230 mesh ASTM), for column chromatography (Merck).

ii. Silica gel 60, particle size 0.063 to 0.200 mm, for column chromatography (Merck).

The gels used were prepared by heating them in a furnace at 500°C for 1 hour, in order to remove any water and plasticiser compounds which may interfere with the final measurements. The gels were subsequently cooled in a dessicator for 12 hours. A desiccant, reagent grade sodium sulphate (Na_2SO_4), was also pre-treated this way. This desiccant comprised the top layer of all columns and removed any traces of water from the sample extracts before they passed through the adsorbent proper.

The columns were then filled with the appropriate amounts of gel and desiccant. Firstly, a glass wool plug was inserted into the neck of the column and the column (including the plug) was then rinsed four times in DCM and subsequently filled with gel. In the case of the alumina columns, 9.6 ± 0.05 g of gel was added, giving a depth of 10 cm. For the silica columns, the same depth of gel was used, but since silica gel has only half the relative density of alumina gel, the 10 cm depth was achieved by using only 4.8 g of gel (± 0.05 g). Finally, a layer of desiccant (0.8 ± 0.05 g) was added to make the top

layer of the column. Gels and desiccant were added to the columns using a clean glass funnel. The sides of each column were then tapped sharply several times with a pencil to consolidate the column contents.

(b):Pure DCM – no significant addition

Firstly, an investigation was carried out to see if the columns themselves were adding any interfering fluorescence to the samples. To do this, one 10 ml sample of pure DCM was passed through each type of column. These two samples were analysed and compared with a sample of pure DCM (not passed through column).

(c):Cleaning of Extracts of pure Loch Lomond water

For the purification process, 6 water samples (900 ml volume) were taken from a site in Loch Lomond in the Field Station Bay (Ordnance Survey Grid Reference NS 377 955). For each type of gel, three samples of pure Loch Lomond water were taken, to see whether background interfering fluorescing materials were effectively removed from the samples.

(d):Retention of Determinands - pure fuel:oil mix in pure DCM.

To ascertain the proportion of the determinands retained after passage through the columns, three samples of pure fuel:oil mixture of 10 $mg.l^{-1}$ in pure DCM of 10 ml volume were passed through each type of column.

All of the aforementioned samples were analysed using the conditions in Table 4.1.

4.3.4.3: Results

a) Pure DCM

Fig. 4.4 shows the amount of emitted fluorescence at 286 nm (excitation λ 262 nm) in the samples of pure DCM before and after passage through each type of column. As can be seen, there is a small increase in emitted fluorescence in the case of both types of gel, but this increase is not very significant, being approximately equivalent to that produced by very low concentrations of volatile aromatic HCs (about 50 $\mu g.l^{-1}$ in DCM).

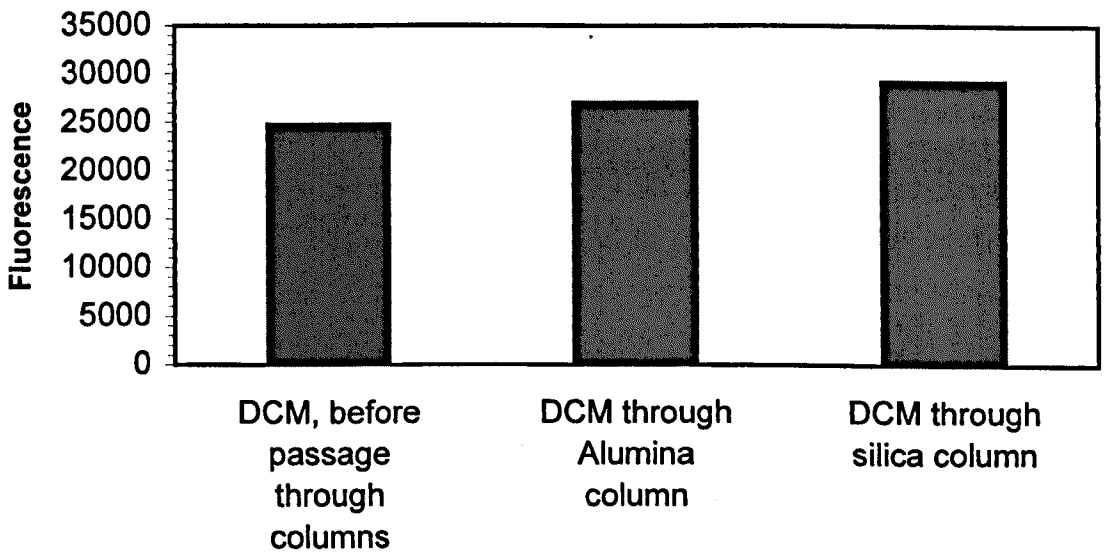

Fig. 4.4: *The fluorescence (in cps) of pure DCM, before and after elution through silica and alumina columns (λ ex. 262 nm, λ em. 286 nm).*

b) Cleaning of Extracts

There was no statistically significant difference between alumina and silica gel in cleaning ability, when the decrease in emitted fluorescence at 286 nm was measured in the cleaned extracts of Loch Lomond water ($t = 0.42$, 2 d.f., n.s.). However, the alumina gel performed slightly better, removing very slightly more of the background fluorescence (mean removal of 23833 cps) than the silica gel (mean of 22567 cps).

c) Retention of Determinands

There was also no significant differences between the retention of determinands by the two different types of columns ($t = 0.48$, 2 d.f., n.s.). Again, the alumina gel performed slightly better, retaining very slightly more of the fuel:oil mixture's fluorescence (mean of 128000 cps, or counts per second) than the silica gel (mean of 121333 cps). Retention of determinands was not high in this preliminary experiment (in both cases, less than 30 %), but the intention here was merely to compare the gels, using exactly the same methods for each. Further experiments in which the method was refined are subsequently described in this chapter (see sections 4.3.6 and 4.3.7).

d) Overall Conclusion

Although the differences between the two types of gel were not statistically significant, both with respect to their ability to remove interfering fluorescence and retain determinands, alumina gel was chosen as it was found to perform slightly better than silica in both respects.

4.3.5: Improving Techniques for the Removal of Background Interfering Fluorescence

Although it was clear that Alumina gel successfully reduced background interfering fluorescence, the best combination of samples size and quantity of gel had not yet been determined. Thus here, a range of gel quantities and sample sizes were tested, to find the optimum method.

4.3.5.1: Methods

Firstly, eighteen water samples (each of 900 ml volume) were taken from a site in Loch Lomond in the Field Station Bay (Ordnance Survey Grid Reference NS 377 955). Three different combinations of purification conditions were investigated, and for each of these, six water samples were taken:

- Three of pure Loch Lomond (LL) water.
- Three of pure LL water, each spiked after extraction with pure fuel:oil mixture to give a total concentration of 10 $mg.l^{-1}$ of fuel:oil mixture in DCM.

Table 4.2 lists the conditions investigated. Unlike in the preliminary purification experiments (see section 4.3.4), a final sample volume was specified here. If the volume of the sample was less than this after elution through the column, more DCM was passed through the column to make up this volume, unlike in the previous experiments (section 4.3.4) where pure DCM was used to make the volume up to that intended. This procedure was intended to increase the proportion of determinands retained and the consistency of retention.

The procedures for column preparation are detailed in Section 4.3.4.2.

Table 4.2: Different column chromatography conditions investigated for their efficacy in the development of sample purification conditions in the current study.

Method	Initial Sample Volume	Weight of Alumina Gel (g)	Final Sample Volume, Eluted Through Column
1	20 ml	4.8 ± 0.05	15 ml
2	10 ml	4.8 ± 0.05	10 ml
3	10 ml	9.6 ± 0.05	5 ml

4.3.5.2: Results

Having investigated the effects of the three combinations of sample volume and gel quantity on sample purification, the best purification method was found to involve 10 ml of sample and 9.6 g of gel and a final sample volume after elution of 5 ml (No. 3 in Table 4.2). Fig 4.5 shows the effectiveness of each of the three conditions analysed in removing interfering fluorescence, while Fig. 4.6 depicts the percentages of determinands retained.

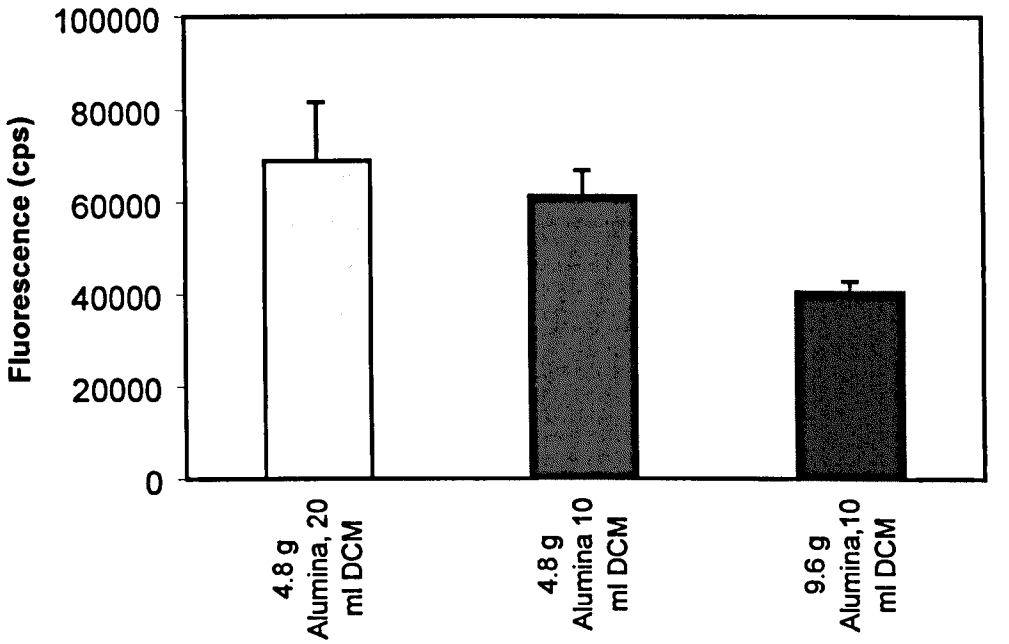

Fig. 4.5: Amount of fluorescence (in counts per second, cps) remaining (λ ex. 262 nm, λ em. 286 nm) after purification of Loch Lomond water sample extracts by the three different combinations of purification conditions, investigated in the current study. Mean of three measurements, and S.D. bars shown.

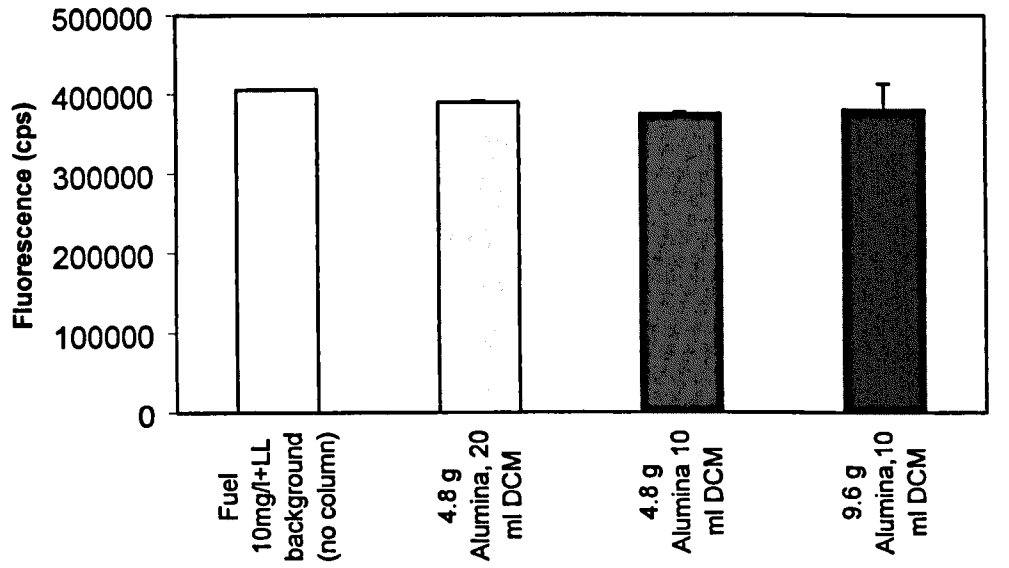

Fig. 4.6: Amount of fluorescence (cps) remaining (λ ex. 262 nm, λ em. 286 nm) after purification of Loch Lomond water samples, spiked after extraction with 10 mg.l^{-1} of 50:1 fuel:oil mixture. Mean of three measurements, and S.D. bars shown. A sample of the unpurified Loch Lomond extract, spiked with 10 mg.l^{-1} of fuel: oil mixture is also shown.

There was no statistically significant difference in the retention of hydrocarbons between the three conditions tested ($F_{2,8}$ = 1.53, n.s., one-way ANOVA). However, there were considerable differences between different conditions with respect to removal of background interference. These differences were found to be statistically significant ($F_{2,8}$ = 9.91, p = 0.013, one-way ANOVA), with the columns containing 9.6g of alumina gel and a final sample volume of 5 ml (after elution) being most effective. These conditions, therefore, were chosen to purify all further samples taken in the present study and are listed in Table 4.3.

More evidence for the effectiveness of this sample purification procedure is demonstrated in Appendix VI.

Table 4.3: Sample purification conditions used in the current study.

Column	9.6 mm I.D.
Stationary Phase	Al_2O_3 90, 9.6±0.1g
Desiccant	Na_2SO_4, 0.5±0.05g
Moving Phase	DCM
Final Sample Volume	5 ml

4.3.6: Linear Range of Calibration

Having established the best wavelengths for excitation and detection (emission), and the best conditions for sample purification, the next stage was to determine the range over which fluorescence emission gives a linear relationship with HC concentration. The first step to achieving this was to produce a calibration curve of fluorescence emission on known HC concentration without the interfering complication of any background fluorescence from substances in the water itself.

4.3.6.1: Methods

Fourteen standards of fuel:oil mixture in pure DCM, with concentrations ranging up to 10 % (w/v) were produced to find the linear range of calibration. This corresponds to concentrations of volatile aromatic HCs of up to 3.26 % (w/v), assuming a proportion of volatile aromatic HCs in petrol of 33.26 %, as calculated in a previous study (Johansen *et al.*, 1983). All of these standards were measured gravimetrically, using a Sartorius Basic B 120 S balance, accurate to 0.0001 g.

4.3.6.2: Results

The relationship between fluorescence (cps) and HC concentration was found to be linear in the range from 0 to 32.59 $mg.l^{-1}$ of volatile aromatic HCs in DCM. At greater concentrations, the increase in fluorescence is non-linear, and at very high concentrations (of over 200 $mg.l^{-1}$ in DCM), the detector response begins to decrease with increasing hydrocarbon (HC) concentration. Fig. 4.7 graphically compares volatile aromatic HC concentration (up to 200 $mg.l^{-1}$) and fluorescence yield, while Fig 4.8 compares these variables up to the highest volatile aromatic HC concentrations of 3.26 % (w/v).

Detector saturation was easy to ascertain, as the fluorescence pattern appeared very different at really high HC concentrations. One obvious difference was the lower excitation peak (at 262 nm). Care was taken that such detector saturation was never wrongly interpreted and samples were diluted (the undiluted sample was kept in reserve) if the slightest chance of high HC concentrations was suspected.

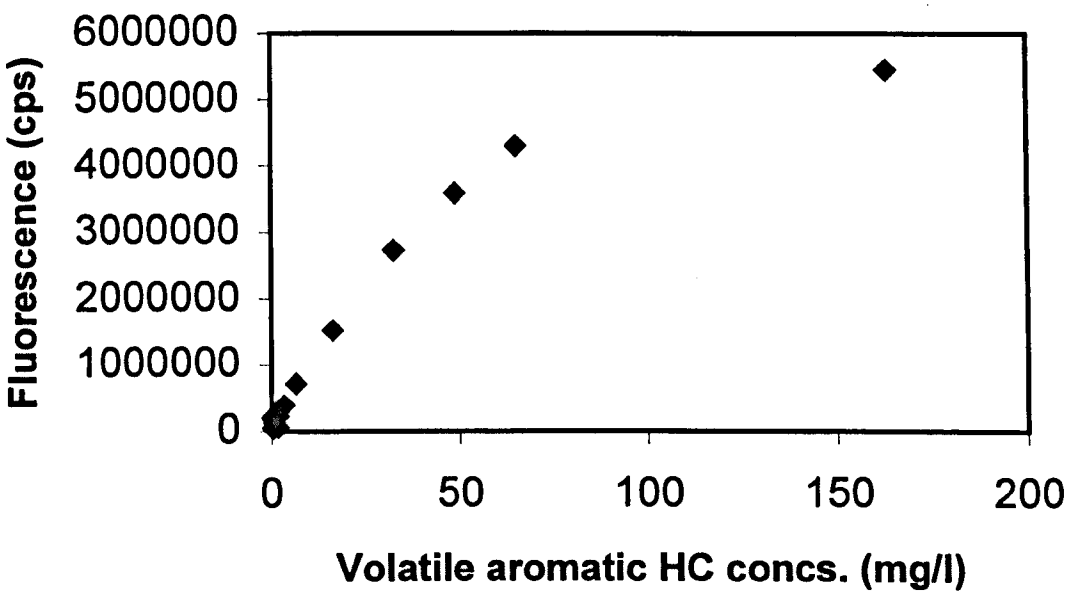

Fig. 4.7: Concentration of volatile aromatic HCs (up to 200 mg.l^{-1}) plotted against total yield of fluorescence (cps), using the method outlined in Table 4.1.

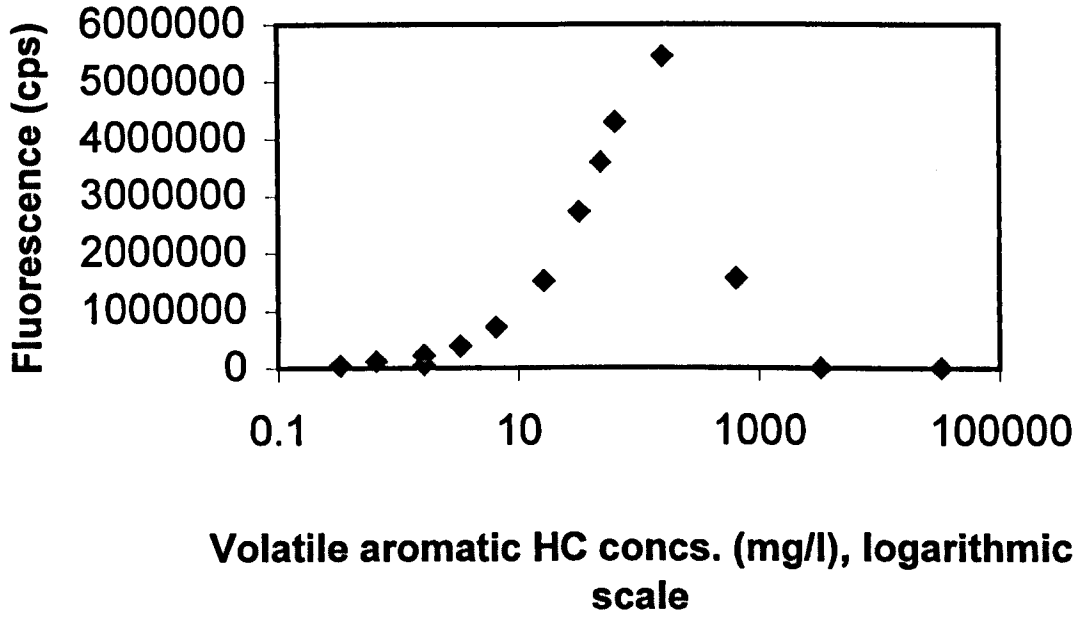

Fig. 4.8: Concentration of volatile aromatic HCs (up to 3.26 % (w/v)) plotted against total yield of fluorescence (cps), using the method outlined in Table 4.1.

4.3.7: Quantification of HCs in water

The next stages in the development of the method required the production of accurate calibration graphs within the linear range of the relationship between HC concentration and fluorescence yield, for the purpose of quantifying volatile aromatic HCs in water.

4.3.7(a): Production of Precise Calibration Graphs

4.3.7(a).1: Methods

In all, two complete calibration graphs were made up for the measurement of volatile aromatic HCs in all of the water samples taken in the present study. For each of these, standards were made up, with concentrations of 50:1 fuel:oil mixture in DCM of 2, 5, 10, 20, 35 and 50 $mg.l^{-1}$. This approximates to concentrations of volatile aromatic HCs of 0.65, 1.63, 3.26, 6.52, 11.41 and 16.30 $mg.l^{-1}$, respectively (assuming a volatile aromatic content of the fuel:oil mixture of 32.59 %). For the purposes of quantification of volatile aromatic HCs in water, these standards correspond to concentrations of 0-90.6 $\mu g.l^{-1}$ in water (using a 180 fold reduction factor, gained by taking 900 ml samples of water with subsequent extraction with DCM and reduction in volume to 5 ml), assuming 100 % efficiency of extraction of HCs from water to DCM. In all, two calibration graphs were produced to analyse the samples in the present study.

4.3.7(a).2: Results

Fig 4.9 shows the two calibration graphs produced in this study. The concentrations of the standards are well within the linear range of calibration and there is little scatter about either of the regression lines:

- $F_{1,7} = 1520.53$, $p < 0.001$, $r^2 = 99.6$ %.
- $F_{1,7} = 2709.14$, $p < 0.001$, $r^2 = 99.7$ %.

The fluorescence method was found to be very sensitive. In a previous study (Law *et al.*, 1988), the detection limits of a similar fluorescence spectroscopy technique were calculated by using the mean background level of fluorescence (at the relevant excitation and emission settings) in the reagent blank, plus three times its standard deviation. All the samples of pure DCM analysed by fluorescence in this study displayed a small but consistent peak at emission wavelength 286 nm with a mean fluorescence count of 22650 counts per second, cps (n = 6, S.D. = 2349). Applying the technique used by Law *et al.* (1988) to the present study gives detection limits of 29697 cps, equating to a figure of approximately 65 $\mu g.l.^{-1}$ of volatile aromatic HCs in DCM or 0.4 $\mu g.l^{-1}$ in water before extraction.

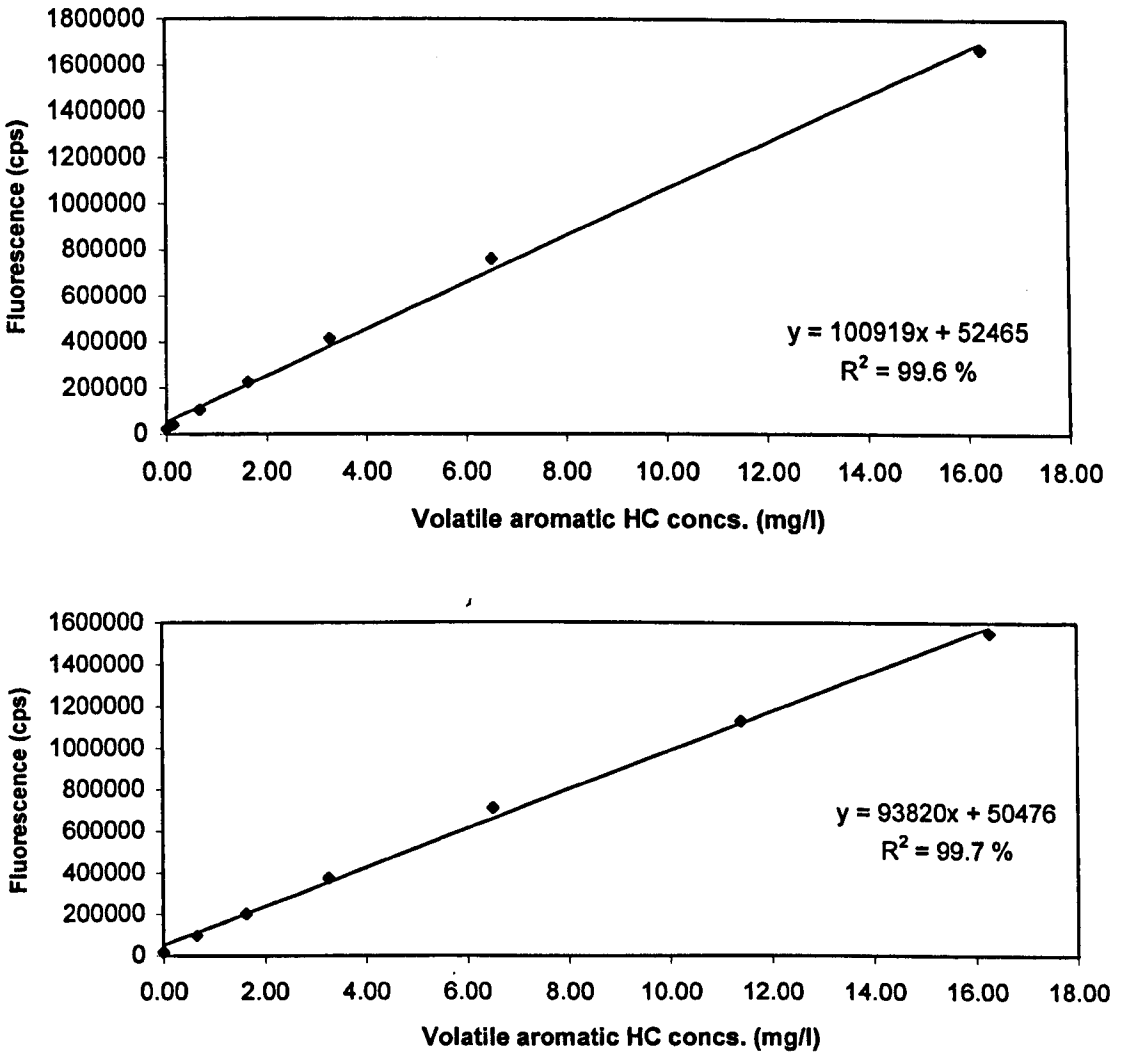

Fig. 4.9: The two calibration graphs, for hydrocarbon measurement in the present study.

4.3.7(b): Extraction Efficiency of Hydrocarbons (HCs) From Water (Fluorescence Analysis)

4.3.7(b).1: Methods

For the measurement of volatile aromatic HC concentrations in freshwaters it is essential to estimate the losses of these compounds at all stages of the sampling and analytical process in the current study, including extraction from water and the subsequent purification of the extracts.

As a first stage in the process, a suitable carrier solvent needed to be found. It had to fulfil several criteria:

1) It needed to be soluble in water
2) It needed to be capable of dissolving the fuel:oil mixture and then delivering this to the water
3) It must not have been capable of causing any interference with the fluorescence measurements of volatile aromatic HCs.

Four different solvents were tested for their efficacy as carriers:

1) Acetone (C_3H_6O)
2) Ethyl-acetate ($C_5H_{10}O_3$)
3) Methanol (CH_4O)
4) Ethanol (C_2H_6O)

Previous studies showed that all solvents chosen were soluble in water to a greater extent than the 1 ml per litre necessary for adding spiking solutions to water. Ethyl acetate was the least soluble, at 100 ml per litre, while all of the other solvents were completely miscible with water (Merck Index, 1996).

The capacity of each solvent to dissolve petrol at an appropriate concentration (50 $mg.l^{-1}$) for a spiking solution was then investigated, by making up solutions of fuel:oil

mixture of that concentration.

Of those solvents which dissolved fuel adequately, their capacity to interfere with the fluorescence spectroscopy analysis was then investigated. For each solvent, 10 ml samples of 500 $mg.l^{-1}$ of pure fuel:oil mixture in pure DCM were made up. To each of these samples were added 10 µl of each of the solvents tested respectively, giving a concentration of each solvent of 1 ppt (w/v) or 1 $g.l^{-1}$ in DCM. In addition, a control fuel standard was prepared in the same way, but without adding any of the solvents tested.

Actual extraction:recovery samples and standards were made up using those solvents remaining which fulfilled all three aforementioned criteria. 1 ml of a solution of pure fuel:oil mixture of 50 $mg.l^{-1}$ (containing approximately 16.30 $mg.l^{-1}$ of volatile aromatic HCs) was added to a standard 1 litre water sample, giving an aqueous concentration of volatile aromatic HCs of 16.30 $\mu g.l^{-1}$. Using standard procedures (see section 3.2.1.3), the HCs were extracted from 900 ml of the water sample into DCM and the volume of the extracts reduced to 5 ml (a 180-fold concentration). This gives a theoretical maximum estimated concentration of volatile aromatic HCs in the extracts of 2.93 $mg.l^{-1}$, assuming 100 % recovery of determinands. To measure the extraction efficiency, an extraction:recovery standard was produced gravimetrically, with a concentration of fuel:oil mixture in DCM of 9 $mg.l^{-1}$ (or 2.93 $mg.l^{-1}$ of volatile aromatic HCs). This corresponds to an extraction:recovery sample with 100 % extraction efficiency.

4.3.7(b).2: Analysis, Including Sample Purification.

All of the calibration standards, including extraction:recovery standards were analysed according to the conditions in Table 4.1, without sample purification. The extraction:recovery samples were analysed in the same way, but also subjected to the sample purification procedure in Table 4.3.

4.3.7(b).3: Results

Of the four solvents, only two (acetone and ethyl acetate) were found to be adequate to dissolve sufficient concentrations of fuel:oil mixture to produce appropriate spiking solutions. Ethanol and methanol did not dissolve the fuel:oil mixture adequately, forming emulsions. The latter two solvents were consequently ruled out as carriers.

Of the remaining two solvents, acetone was found to be inappropriate, as it extinguishes almost all of the fluorescence from the fuel:oil mixture, possibly due to a very high absorption of the excitation light. Ethyl acetate had no such adverse effects and it was chosen as a carrier solvent in the present study.

Altogether, ten extraction recovery samples and standards were produced in the current study. Recovery of fuel:oil mixture volatile aromatic HCs was good, with a mean of 86.40 % (standard deviation 13.01 %).

4.3.8: Comparison of Fluorescence Technique with GC-MS

One final test of the efficacy of this modified method was to compare the results obtained from fluorescence spectroscopy with those using a more established (but more laborious) technique: GC-MS (see Sections 3.2.1.4 and 4.3.9 and also Appendix I for more details of the method).

4.3.8.1: Methods

Following the sampling programme of 1996, 8 of the 105 samples (taken from Loch Lomond during the summer of 1996) which were analysed by Fluorescence Spectroscopy (see Chapter 5 for details of the samples taken) were subjected to a subsequent confirmatory analysis by GC-MS. The analytical conditions are shown in

Table 3.1, but with one difference. Instead of scanning a broad range of ions, selective ion recording was used here, with only ions of mass 78, 91, 105, 119, 120, 128, 133, 134, 142 a.m.u. monitored.

For the purposes of the comparison, only 2 monocyclic aromatic hydrocarbon (HC) compounds (1,2- and 1,3-dimethyl-benzene) were quantified. However, these compounds comprise 16 % of the water soluble aromatic hydrocarbons in powerboat exhaust (see Chapter 3) and it was assumed that the concentrations of these two compounds would be a good indicator of the total quantity of unburned fuel hydrocarbons emanating from powerboats.

4.3.8.2: Results

Fig. 4.10 shows the relationship between the concentrations of the two known standard HC compounds, measured by GC-MS and the total volatile aromatic HC concentrations (equivalent to the total amount of fuel:oil mixture), measured by fluorescence spectroscopy, in the eight samples selected for this paired analysis. These concentrations are those measured in the sample extracts and extraction efficiencies were not taken into account. A reasonably strong statistical relationship was gained ($F_{1,7}$ = 22.52, p = 0.003, r^2 = 75.5 %), indicating that the concentrations of the known HC compounds (measured by GC-MS) were a reasonably accurate predictor of total volatile hydrocarbon concentrations, measured by fluorescence spectroscopy.

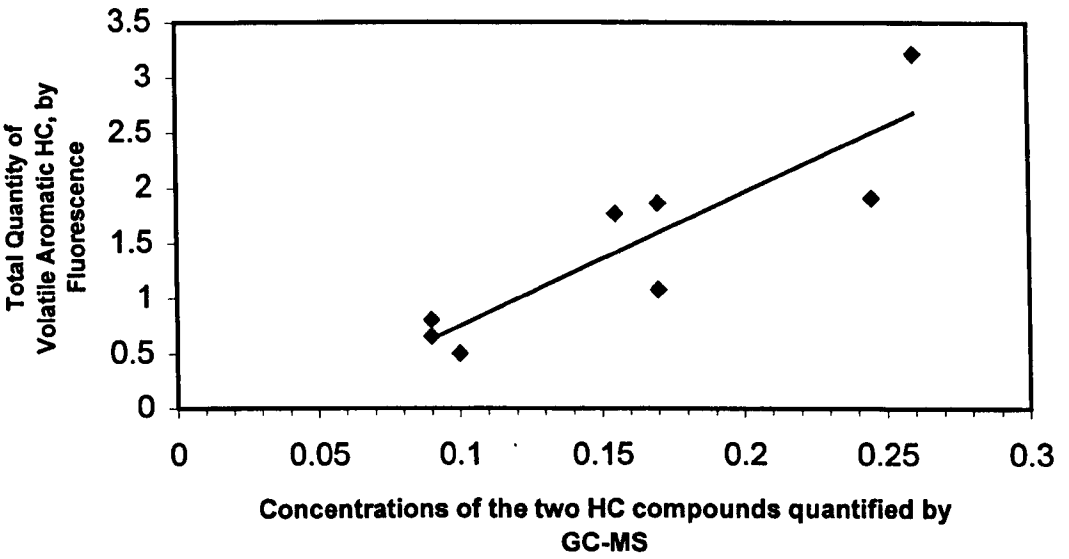

Fig. 4.10: Comparison of hydrocarbon measurements by the fluorescence and GC-MS methods used in the present study.

4.3.9: Efficiency of Extraction of Particular HC Compounds from Water (for the purposes of GC-MS analysis)

To quantify volatile aromatic HCs in water by GC-MS, just as in fluorescence analysis, sample processing losses need to be known.

4.3.9.1: Methods

In all, six extraction:recovery samples were made up with seven compounds examined for their extraction efficiency in each of these six samples:

- Benzene.
- Methyl-benzene (Toluene).
- 1,3 dimethyl-benzene.
- 1,2 dimethyl-benzene.
- 1,3,5 trimethyl-benzene.

- Naphthalene.
- 1-methyl-naphthalene.

To produce these extraction:recovery samples, a spiking solution was made up by diluting each of these compounds at a concentration of 50 $mg.l^{-1}$ in ethyl acetate, using a Sartorius Basic B 120 S balance, accurate to 0.0001 g. 1 ml of this spiking solution was then added to a 1 litre sample of water from a depth of 1.5 m at a site in Loch Lomond subject to very low powerboat activity. In addition, a reference sample was taken from that depth at the same site. This sample was not spiked in order to measure the background levels of volatile aromatic HCs.

Both the reference samples and the extraction:recovery samples were subsequently extracted using standard methods (see Section 3.2.1.3). The extracts were then reduced in volume to 5 ml, and the samples analysed using the conditions in Table 3.1. There was one difference in the sample processing and analytical procedure used in this part of the study: only a limited range of ionic fragments, with masses 78, 91, 105, 119, 120, 128, 133, 134, 142 a.m.u. were monitored. This " selective ion recording" (SIR), which is far more sensitive than scanning ion recording, producing a much greater MS response for a given concentration of a compound. Thus, a range calibration standards of lower concentration were produced for this particular sample set, with each of the aforementioned compounds diluted in DCM at concentrations of 40, 100 and 200 $\mu g.l^{-1}$, in addition to a DCM reagent blank. These standards are approximately fifty times lower in concentration than those described in Section 3.2.1.5 and the sample extracts were correspondingly diluted fifty fold prior to analysis.

The extraction efficiency in each of the six samples was measured by producing an extraction:recovery standard, with concentrations of the aforementioned compounds of 180 $\mu g.l^{-1}$. This is equivalent to the concentration of these compounds in an extract of one of the extraction:recovery standards following extraction and subsequent dilution, assuming 100 % recovery of determinands and background levels of these compounds (in the reference samples) of zero. The extraction efficiency of each compound in each

extraction:recovery sample was calculated as:

[(x-y)*100]/180, expressed as a percentage, where:

x = concentration of the compound in the extraction:recovery sample.

y = concentration of the compound in the reference sample.

4.3.9.2: Results

The extraction efficiencies for six aromatic HC compounds are listed in Table 4.4.

Table 4.4: Extraction efficiency of six aromatic HC compounds in the present study; mean, range and S.E. of six samples.

Compound	*% Retention*	*S.D.*
Benzene	46.34	28.25
1,3-Dimethyl-benzene	56.99	17.11
1,2-Dimethyl-benzene	49.07	14.59
1,3,5-Trimethyl-benzene	34.86	9.22
Naphthalene	37.22	14.21
1-Methyl Naphthalene	44.33	16.53
MEAN	44.80	14.43

4.4: Discussion

4.4.1: Effectiveness of the Modified Fluorescence Spectroscopy Method

The fluorescence-based method described here has been successfully used to detect and measure aqueous concentrations of volatile aromatic hydrocarbons (HCs) in Loch Lomond as low as 0.4 $\mu g.l^{-1}$. To date, no other method based on this technique has been used to measure such low (as low as background environmental) concentrations of volatile aromatic HCs. Fluorescence spectroscopy is typically a qualitative technique, used to distinguish different types of crude oils (see John and Soutar, 1981). In only one previous study have such methods been used to measure monocyclic aromatic HCs (Siron *et al.*, 1991) and even then only for measuring relatively high aqueous concentrations (above 0.2 $mg.l^{-1}$). The excitation (262 nm) and emission (287 nm) wavelengths in that previous study were almost identical to those of the present study but further details about the method are lacking.

One of the reasons for the sensitivity of the fluorescence-based method in the present study is the sample purification procedure. Such techniques have been used previously, but for very different reasons. In the analysis of marine oil spills, following preliminary identification using fluorescence, samples are only purified before more detailed chromatographic analysis (GC-MS or HPLC: see Law *et al.*, 1988). By contrast, the purification process in the present study has been successfully used to greatly improve the sensitivity of the fluorescence-based method and chromatographic techniques, when used in a subsequent confirmatory analysis, showed a high degree of correlation between the techniques. Thus, this technique provides an accurate rapid, relatively simple and highly cost-effective method for the detection of hydrocarbon (HC) compounds in freshwater.

4.4.2: Limitations of the Fluorescence-Based Technique

The modified fluorescence method in the current study was found to be highly sensitive, and the calibration graphs produced suggest that precision is also high. Some basic future research would be useful, however, to more accurately determine the capabilities of the method. This would include quantitative determination of precision and bias and confirmation of the detection limits. Standard procedures for such refinement of the method are given in Cheesham and Wilson (1989).

The fuel:oil mixture, used to calibrate the spectrofluorimeter, consisted of "Thames" regular unleaded petrol and "Castrol" two-stroke motorcycle oil. In this mixture, only the aromatic compounds fluoresce, and this fluorescence can only indicate the presence and quantity of these compounds in a sample. Previous research has shown that petrol (and consequently the fuel:oil mixture used in the present study, which is 98 % petrol) consists mainly of aliphatic compounds. These compounds do not fluoresce, and although they comprise the majority of the HCs in the aforementioned standards, their presence in a sample cannot be inferred by the fluorescence of the volatile aromatics.

Owing to their insolubility in water, aliphatic compounds are also very unlikely to be found in water in any significant quantities (Peterson, 1994). This has been confirmed in previous research, both in the current study (Ch. 3) and previous studies (Montz *et al.*, 1982; Jüttner, 1994; Jüttner *et al.*, 1995*a*). All of these studies have shown that the compounds from outboard motor (2- and 4-stroke engines) exhaust entering water almost exclusively consist of volatile aromatic HCs.

In order to accurately measure volatile aromatic HCs in powerboat (particularly outboard motor) exhaust-polluted water (EPW), using the uncombusted 50:1 unleaded fuel:oil mixture as a calibration standard, it was necessary to know the proportion of volatile aromatics in the fuel:oil mixture. Unfortunately, this was not carried out in the current study and the proportion of aromatic HCs in all standards in the present study

needed to be estimated from a previous study. Johansen (1983) analysed four different samples of regular unleaded petrol (from four different service stations) and found that the mean volatile aromatic content was 33.26 %. Since lubricant oils are relatively non-volatile (NRC, 1985) and make up only 2 % of the fuel:oil mixture, it was assumed that they did not contribute to the total amount of volatile aromatics in the fuel:oil mixture. Assuming that the mean aromatic content of petrol measured by Johansen (1983) is similar to the actual (but unknown) aromatic content of the unleaded petrol in the current study, this gives an estimated aromatic content of the 50:1 fuel:oil mixture (used in the current study) of 32.59 %. An sample extracted from water with fluorescence measurements equivalent to 10 mg.l-1 of fuel:oil mixture in DCM would therefore possess 3.26 mg.l-1 of volatile aromatics in DCM and 18.11 $\mu g.l^{-1}$ in water (assuming an 180 fold reduction in sample volume).

The estimated proportion of volatile aromatic HCs in the fuel:oil mixture may be slightly inaccurate, but since the same stock sample was used to calibrate the spectrofluorimeter throughout the current study, any errors were consistent throughout the study, and the main conclusions would not have been altered if the actual proportion of aromatic HCs was known.

In the preliminary GC-MS analysis of artificially polluted water (see Chapter 3, Section 3.3.1), only traces of aliphatics were detected, possibly due to their very low aqueous solubility. Although aliphatic compounds do not contribute significantly to the total quantity of volatile HCs in powerboat exhaust polluted water (EPW), they may contribute a significant proportion of the toxicity of petroleum oils, despite their low solubility, as they can be toxic at concentrations within the range of their aqueous solubility (Peterson, 1994; NRC, 1985). It is conceivable that, although the measurements of the total quantity of water-soluble HCs in this study are reasonably accurate, the toxicity of powerboat EPW may be higher than that expected from the aromatic content alone.

4.4.3: The Fluorescence Spectroscopy: GC-MS Comparison

In the comparison of GC-MS and fluorescence analyses, the extraction:recovery efficiencies were not taken into account. This was due to the surprising difference in extraction efficiency readings gained from the two methods. A mean measurement of 86.4 %, was gained using the fluorescence technique (see Section 4.3.7(b).3) but only 44.8 % (mean for six compounds), using GC-MS (see Section 4.3.9). This difference was found to be highly statistically significant ($U = 113.0$, $p = 0.0028$, $n = 10$ for fluorescence and 6 for GC-MS; Mann-Whitney U-Test). It therefore seems that there could have been differences in the behaviour of the determinands during the process of making up extraction:recovery samples, possibly due to the different spiking solutions used for the different analytical methods. Previous studies have shown that the dissolution of complex mixtures of HCs is indeed a complicated procedure (Shiu *et al.*, 1988; Peterson, 1994). Further research of a more fundamental nature is necessary to investigate the behaviour of different mixtures of organic compounds in water.

There may be two other reasons for the low extraction efficiency of the volatile aromatic HCs by GC-MS:

- GC-MS analytical conditions were most appropriate for pesticides, which are more highly polar and much heavier (and less volatile) than volatile aromatic HCs.
- The extraction:recovery standards were produced by dissolving the HCs in a carrier solvent and delivering this spiking solution to the water. This process may not reflect the "real life" situation of entry of HCs to the water from powerboat exhausts.

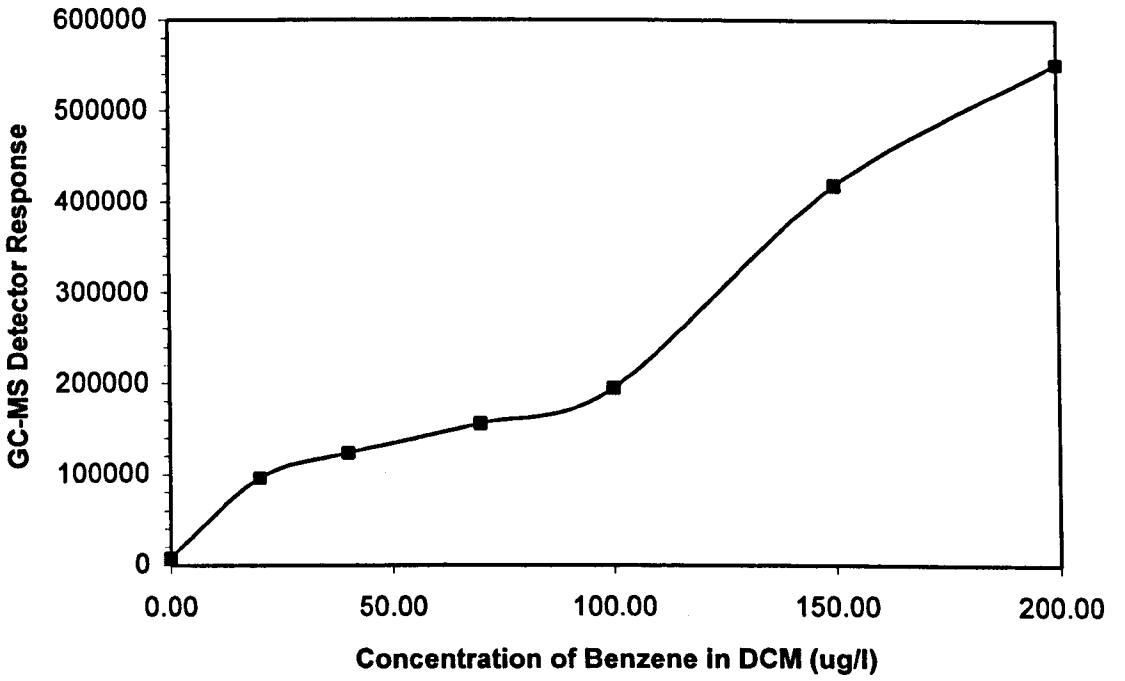

Fig. 4.11: Example of a calibration graph (with interpolation) used in GC-MS analysis in the present study. The compound calibrated is benzene.

As previously mentioned, a reasonably strong statistical relationship was found in the regression analysis of the concentrations of volatile aromatic HCs measured by fluorescence spectroscopy versus the concentrations of the two compounds measured by GC-MS. There is some scatter about the regression line, but this may have been due to the fact that only two compounds were quantified in the GC-MS measurements of HCs in the current study. These compounds comprised 16 % of the organic compounds emanating from powerboat exhaust and were found to be a reasonably accurate predictor of the total concentrations of volatile aromatic HCs measured by fluorescence spectroscopy in the eight samples compared. Four other compounds were measured, but these were only detectable in a few of the samples. Unfortunately, toluene (by far the most abundant aromatic compound, both in petrol and EPW) could not be quantified, due to atmospheric contamination of the laboratory at the time of this analysis (December 1996 to January 1997). This was not the case in preliminary studies (see Ch. 3). However, the fuel:oil mixture contains hundreds more different compounds (Johansen *et al.*, 1983)

and it is possible that greater predictive accuracy would be achieved in the comparison if more compounds were quantified by GC-MS.

It was also slightly more difficult to quantify compounds, using GC-MS than it was by fluorescence. Fig. 4.11 shows a calibration graph for benzene, one of the compounds quantified. Since the relationship is non-linear and not feasibly transformable to linearity, the only satisfactory way of calibration involved interpolation (demonstrated in the graph). All of the other compounds also demonstrated this characteristic to some extent and this could have led to inaccuracy in the comparison of the two methods.

Another reason for the less than perfect relationship between the results of the two methods could have been different proportions of the HC compounds present in the water samples. There are a wide variety of fuel and lubricant oil types discharged by boats on Loch Lomond and although outboard motors are responsible for the vast majority of HC pollution in Loch Lomond, pollution from boats powered by inboard petrol and diesel engines also occurs (see Table 2.6). There may have been some compositional variation in the samples, due to the corresponding variation in the proportions of different fuel types and lubricant oils in the water samples before extraction. These oils themselves may have varying proportions of different volatile aromatic HC compounds. A potential improvement in the method might involve ascertaining the proportion of different types of petroleum oils in water samples from Loch Lomond.

Chapter 5

The Temporal and Spatial Distribution of Powerboat Exhaust Hydrocarbons in Loch Lomond

5.1: Introduction

Although there is a considerable body of published literature on hydrocarbon (HC) pollution, few studies have yet been carried out in which the link between powerboat exhaust emissions and freshwater pollution has been investigated. Only one such study (in addition to the current study) exists to date (Miller and Fiore, 1997) and in that study, volatile aromatic hydrocarbons (HCs) were monitored in Lake Tahoe, California, USA. It was found that powerboat operation increases the concentrations of these compounds. The environmental fate of these compounds in Lake Tahoe was also briefly examined.

In the current study (Ch. 3), it has already been shown that powerboat exhaust HCs can be detected in Loch Lomond. In order to carry out further, more detailed investigations, relatively large numbers of water samples needed to be analysed. This

necessitated the development of a new fast, accurate and reliable method for the analysis of volatile aromatic HCs (which comprise the bulk of the substances entering water from powerboat exhausts, see Jüttner, 1994 and Mele, 1993) in freshwater. Details of the development of this method are found in Ch. 4.

Another pre-requisite in such a large-scale survey was a reliable method of water sampling, in which the risk of contamination of samples was kept to a minimum. Such a method is described in the current chapter and in addition to this, a brief experiment was carried out, in order to check the validity of the sampling method.

The combination of a new method of water sampling and analysis allowed the completion of hitherto unfeasible investigations of powerboat hydrocarbon pollution in Loch Lomond. Here, I use these techniques to examine:

1) The geographical distribution of powerboat exhaust HCs in Loch Lomond.

From earlier work in the current study (Ch. 3, Section 3.3.3), HC compounds originating from powerboat exhaust were detected in the surface waters of Loch Lomond at three sites on a weekend day of high powerboat activity in summer. To investigate this further, the spatial distribution of surface water powerboat exhaust HCs was ascertained by taking surface water samples from many sites in Loch Lomond on a similar summer weekend day.

2) The depth profile of powerboat exhaust HC compounds in the water column.

In the current study (Ch. 3, Section 3.3.1), it was shown that the main components of outboard motor exhaust entering the water were volatile aromatic HCs. Traces of aliphatic HCs and Polycyclic Aromatic Hydrocarbons (PAHs) were also found. In a previous study (Wachs *et al.*, 1992), it was found that aliphatics from powerboat exhaust were primarily found at the water surface microlayer (the depth of this was not specified).

In another study (Hardy *et al.*, 1990), it was shown that PAHs (from a variety of sources) were largely confined to the surface microlayer (the top 60 μm) of the water column (in Chesapeake Bay, USA). In the current study, it was intended to see whether or not volatile aromatic HCs from powerboat exhaust behave in this way by investigating the vertical distribution of these compounds in the top 1.5 m of the water column.

3) Recovery of water quality from powerboat pollution.

The results of boat censuses have shown that mean weekday afternoon numbers of powerboats on Loch Lomond drop to well under half of those present at the weekend (Adams *et al.*, 1992; Adams and Grant, 1997) and it was consequently thought that there could be recovery in water quality from HC contamination from powerboats during the course of a summer week. The current study aims to investigate the potential for such recovery by taking surface water samples from a suitable site in Loch Lomond throughout the course of a summer week.

To complement the field studies of both depth profile of powerboat exhaust HCs and recovery of water quality from HC contamination, suitable controlled experiments were carried out.

5.2: Aims

1) To ascertain the spatial distribution of surface water HCs in Loch Lomond.
2) To plot the vertical distribution of HCs in the topmost 1.5 m of the water column.
3) To investigate recovery of water quality at an appropriate site in Loch Lomond, following heavy recreational boating activity.
4) By means of controlled experiments, to further investigate powerboat exhaust HC pollution in freshwater. This included studies of:
   i. the introduction of volatile aromatic HCs to the water.
   ii. the proportion of fuel used that remains in the water.
   iii. the depth profile of volatile aromatic HCs in the water column following an artificial pollution episode.
   iv. the recovery of water quality following powerboat pollution.
5) To assess the validity of the water sampling procedure used in the present study.

5.3: Methods

5.3.1: Methods for Water Sampling and Hydrocarbon Analysis

5.3.1.1: Preparation of Glassware

The same procedures for cleaning and drying glassware described in Ch. 3, section 3.2.1.1 were used for the experiments described in the current chapter. These procedures were effective, not only in removing contaminating HCs but also any other substances, such as humic acids, lipids and amino acids, that could interfere with the analytical techniques used.

5.3.1.2: Experimental Methods: Surface Water Sampling

Surface water samples were collected by holding the opened glass bottles carefully at the water surface and allowing them to fill slowly. The procedure is described in more detail in Ch. 3, Section 3.2.1.2.

5.3.1.3: Experimental Methods: Sub-surface Water Sampling

Sub-surface water sampling devices were constructed in such a way that only clean glass touched the water at all times during sampling. The devices consisted of glass separating funnels, with taps wedged open, securely connected to glass tubing and sealed on the outside by waterproof insulating tape. The glass tubes were closed at the top by means of tightly fitting rubber stoppers and support for the devices was provided by means of metal or wooden frames. Fig. 5.1 depicts one of these devices.

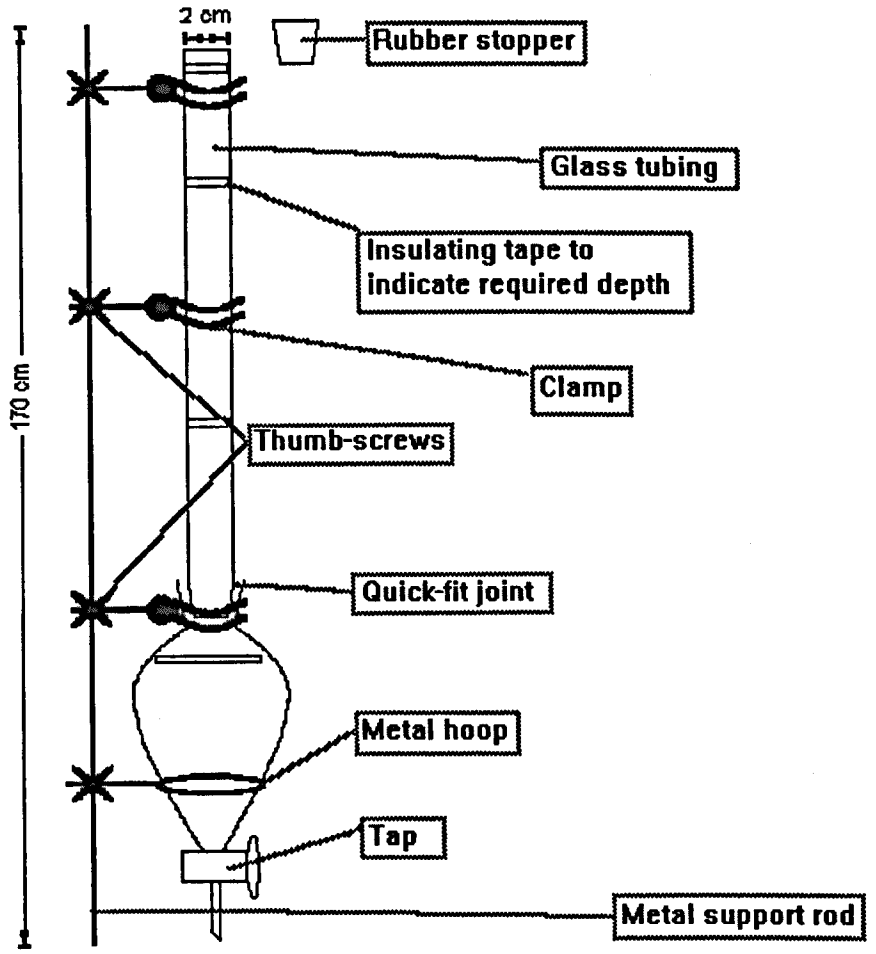

Fig. 5.1: Device used to take sub-surface water samples in the current study.

The device was deployed by lowering it over the side of the sampling boat (in the field experiments) or into the experimental tank (in the controlled experiments) in the closed position (i.e. with the rubber stopper in place) until the required depth was reached. This was indicated by depth markers, on the sides of the glass tubing. The rubber stopper was then taken out and the devices allowed to fill with water, which could only enter the device from the opened tap, which itself was at the required depth. Once the sampling device was full, the rubber stopper was carefully replaced and the device raised up into the boat, the stopper removed and the water sample poured into a clean sample bottle. The water was then allowed to degas, as previously described for surface water samples (see Section 3.2.1.2) and the lid fitted.

The device was only used after being thoroughly washed as described in Section 5.3.1.1. Since the washing and drying procedure took 24 hours and five samples (from different depths) were taken on each day of sampling, it was necessary to construct five of these devices.

5.3.1.4: Experimental Preparation, Controlled Experiments

A series of controlled experiments were carried out in a 1787 litre stainless steel tank. Before each experiment, the inner surfaces of the tank were cleaned very thoroughly, first by removing excess dirt and dust with a nylon scourer and tap water, delivered from a high-pressure hose. Hydrocarbon residues were then removed by wiping the inner surfaces of the tank with paper towels soaked in acetone. The acetone was removed by washing the tank very thoroughly 5 times in tap water, using a high-pressure hose. Finally, the tank was again thoroughly rinsed (also 5 times) with the water used to fill it for experiments, which was taken from the Field Station's untreated experimental supply, which is extracted from a depth of 2.5 m in the Field Station Bay of Loch Lomond, 100 m WSW of the jetty (Ordnance Survey Grid Reference NS 377 957).

Prior to engine operation in all experiments, two control water samples of 1 litre volume were taken. The first was taken as soon as the tank was filled and the second just after the engine was placed in the tank, to see if any residual HCs entered the water from the engine's exhaust system. The relatively high volume of sample was necessary, as the concentrations of HCs were expected to be low.

5.3.1.5: Sample Processing

Following collection, all water samples were stored in a refrigerator at 4°C for a maximum period of 48 hours before extraction of hydrocarbons. All samples were then liquid:liquid extracted using dichloromethane (DCM) and the extracts reduced in volume to 1 ml (for the validation experiment, described in Section 5.3.2.1) and 5 ml (for all other experiments). The extraction procedure is described in more detail in Section 3.2.1.3. Sample processing losses were estimated using the procedure in Section 4.3.7(b).

5.3.1.6: Analytical Methods

Following the sample extraction and reduction process (Section 5.3.1.5), the samples (containing the HCs) were analysed by fluorescence spectroscopy, with an excitation wavelength of 262 nm. and emission wavelength of 286 nm (see Table 4.1), following sample purification by normal-phase column chromatography (see Table 4.3). Appendix I gives more detail of the HC analytical methods used.

In the validation experiment (Section 5.3.2.1), samples were analysed by Gas Chromatography-Mass Spectrometry (GC-MS), using the conditions outlined in Table 3.1, with one exception, whereby ion masses from 20 to 420 a.m.u.s were analysed.

5.3.2: Specific Experimental Methods

5.3.2.1: Verification of the Water Sampling procedure

In order to see whether or not the motor boat used for water sampling was itself causing contamination of samples taken on Loch Lomond, an investigation was carried out on a calm day (May 6 1996) at 9.00 am (a time of very low boat activity - see Bannan *et al.*, 1995) at a site where boat numbers are known to be always low: 400m SE of Ross Point, Ordnance Survey Grid Reference NS 372 950. This site is located in Boat Census Survey Area No. 6 (see Ch. 2, section 2.2.1.1).

Surface water samples were taken from appropriate locations around the sampling motor boat and downstream from the exhaust outlet of the engine, viz.:

1) A control sample, taken at the sampling site using a row-boat, before commencement of engine operation.
2) An extraction:recovery sample taken at the same time and place as No. 1, to measure sample processing losses.
3) A sample taken from the starboard side of the vessel, equidistant from the bow and the stern of the motor boat.
4) A sample taken from the port side of the vessel, equidistant from the bow and the stern of the motor boat.
5) A sample taken from directly behind the exhaust outlet, astern of the motor boat.
6) Pure DCM – solvent blank.

Different methods of sample processing and analysis were used to those employed in other experiments described in the current chapter. Hydrocarbons were solid-phase extracted from 800 ml of each 1 l water sample, using a "Zymark Autotrace" and the extracts were reduced in volume to 5 ml, using a "TurboVap", automatic sample concentration device.

Three standards were produced, with concentrations of 2, 10 and 50 $mg.l^{-1}$ of each of the following compounds, diluted in DCM:

- Benzene.
- Methyl-benzene (Toluene).
- 1,3 dimethyl-benzene.
- 1,2 dimethyl-benzene.
- 1,3,5 trimethyl-benzene.
- Naphthalene.
- 1-methyl-naphthalene.

Sample processing losses were measured by the procedure outlined in Section 4.3.9, except that acetone, instead of ethyl-acetate was used as the carrier solvent to deliver the aforementioned HC compounds to the water sample before extraction. An extraction recovery standard was also made up, with concentrations of the standards in DCM of 2 $mg.l^{-1}$, equating to a 160-fold increase in concentration of each compound, which was present in the extraction:recovery sample at an aqueous concentration of 12.5 $\mu g.l^{-1}$. A concentration of 2 $mg.l^{-1}$ in the extracts equates to the theoretical maximum 100 % recovery of the HC compounds in the extraction:recovery sample (assuming no background contamination of the water before it was spiked).

All sample processing and analysis in this particular experiment took place at the laboratories of the Scottish Environmental Protection Agency (SEPA), West Region, East Kilbride, Scotland.

5.3.2.2: Geographical Distribution of Powerboat Exhaust Hydrocarbons in Loch Lomond

(a): General Experimental Procedures

On July 21 1996, surface water samples of 1 litre volume were taken from 22 sites on Loch Lomond, to investigate the spatial distribution of powerboat exhaust HCs. These sites are listed in Table 5.1 and indicated on the map displayed in Fig. 5.2 and were pre-selected on the basis of known patterns of boat activity on Loch Lomond (see Adams *et al.*, 1992; Adams and Grant, 1997). Local boat densities (for the particular boat census survey area – see Section 2.2.1.1) at each site are also listed in Table 5.1 (column 4); data from Adams and Grant (1997).

Table 5.1: Sites on Loch Lomond from which water samples were taken to investigate the geographical distribution of surface water volatile aromatic HCs (also indicated on Fig. 5.2). Mean (of 15 censuses) summer weekend powered boat densities, measured in the boat census survey areas in which each site is located are also shown. Data from Adams and Grant (1997).

Site No.	Site	Boat Census Survey Area	Boat Density in Each Area (boats per sq. km)	Ordnance Survey Grid Reference
1	Field Station Bay	6	4.11	NS 377 957
2	Area 6 Open Water Site	6	4.11	NS 372 950
3	Milarrochy Bay Outer	1	12.17	NS 409 924
4	Milarrochy Bay Inner	1a	162.05	NS 411 924
5	Balmaha Outer Moorings	2	2.25	NS 418 907
6	Balmaha Pier	2	2.25	NS 419 909
7	Area 3 Open Water Site	3	8.49	NS 377 844
8	Cameron House	3	8.49	NS 376 832
9	River Leven Exit	3	8.49	NS 386 825
10	Area 4 Open Water Site	4	3.92	NS 385 894
11	Inchmoan S. Beach	4	3.92	NS 373 907
12	Haven	5	46.23	NS 371 910
13	Channel between Inchmoan and Inchconnachan	5	46.23	NS 376 911
14	Narrows Outer	5	46.23	NS 371 912
15	Narrows Middle	5	46.23	NS 371 914
16	Narrows Inner	5	46.23	NS 372 916
17	Luss Outer	6	4.11	NS 363 933
18	Luss Inner	6a	193.85	NS 362 931
19	Inverbeg Campsite Moorings	8	5.46	NS 346 985
20	Area 10 Open Water Site	10	2.00	NN 332 060
21	Area 12 Open Water Site	12	17.94	NN 320 155
22	Ardlui Marina	12	17.94	NN 318 156
	WHOLE LOCH		5.32	

For the purposes of this experiment, two extra boat census survey areas were designated, to more precisely measure the local boat densities at two of the sites from which water samples were taken (for HC analysis). From informal observation during boat censuses carried out prior to 1996, it was felt that survey Areas 1 and 6 were too large and heterogeneous for their mean density to have any bearing on HC concentrations

at sites 4 and 18 respectively, which are both located in areas of locally heavy powerboat activity within each of census survey areas 1 and 6 respectively. The two new census areas were sub-divisions of Area 1 and 6, and called Areas 1a and 6a respectively (see Fig. 5.3). The data from the boat censuses was used to see if local boat densities could predict surface water HC concentrations.

Water samples were also taken from five open water sites with consistently low boat numbers (known from previous censuses – see Adams *et al.*, 1992 and Adams and Grant, 1997). These sites were representative of most of the area of Loch Lomond, which has relatively low powerboat activity. Also, from a reference site (No. 2 – see Table 5.1), samples were taken from a depth of 1.5 m on almost every occasion on which samples were taken during the summer of 1996. In all, nine such reference samples were taken, to further investigate background levels of powerboat exhaust pollutants in Loch Lomond. Site 2 was deliberately chosen in an area known (from informal observations) to have very low powerboat activity.

All of the other sites chosen were deliberately located in areas of relatively high powerboat activity, to attempt to gain as much information as possible (from the number of samples taken) about the distribution of powerboat exhaust contaminants in the surface waters of Loch Lomond. Three of the sites, including two of high (Nos. 6 and 16) and one of low boat activity (No. 10), were sampled in the pilot study and the results are detailed in Chapter 3, section 3.3.4.

All water samples were taken concurrently with a boat census survey, carried out between 12 noon and 5 p.m. Standard methods (see Adams *et al.*, 1992) of boat counting were used, but with the exception that the aforementioned new survey areas (Areas 1a and 6a) were included.

In one of the analyses of data from the experiment described in this section, each data point represented the local boat density in that area (x axis) compared to the measured surface water volatile aromatic HC concentration in each survey area sampled (y axis). When more than one water sample was collected for hydrocarbon analysis from a particular survey area, the mean hydrocarbon concentration for that particular census survey area was calculated. This gave one data point for each of the 11 census survey areas (out of a total of 14 in the whole of Loch Lomond) from which water samples were taken for HC analysis on July 21, 1996.

(b): Estimation of Sampling Variation

Since replicate samples were not taken at each site in this experiment, an alternative method was necessary to estimate sampling error. Of the 22 sites sampled, 7 were designated as having "high" levels of active powerboats, 6 had "moderate" levels while the other 9 had "low" levels. Table 5.2 defines these categories, with mean, median, range and S.E. given, with all values in boats.km^{-2}.

Table 5.2: The three designated categories of powerboat activity (boats.km^{-2}) on July 21 1996.

Designation	n	Mean	Median	S.E.	Range
Low	9	4.2	5.5	0.6	1.2–5.6
Moderate	6	9.7	9.3	0.7	7.0-11.6
High	7	114.6	43.1	48.4	43.1-360.0

Evidence for the validity of these groupings was gained by performing a Kruskal-Wallis one-way ANOVA. It was found that there was a statistically significant difference between the medians of the groups designated ($H = 18.83$ d.f. $= 2$ $p = 0.000$), with respect to their powerboat density.

It was presumed that the levels of powerboat activity in each of these groupings would in turn reflect the levels of volatile aromatic HCs in the samples within the groups. To gain an indication of the sampling variability of HCs at each site, the S.E. of the HC

concentrations within each of the above groups (based on the levels of powerboat activity) was used for each sample.

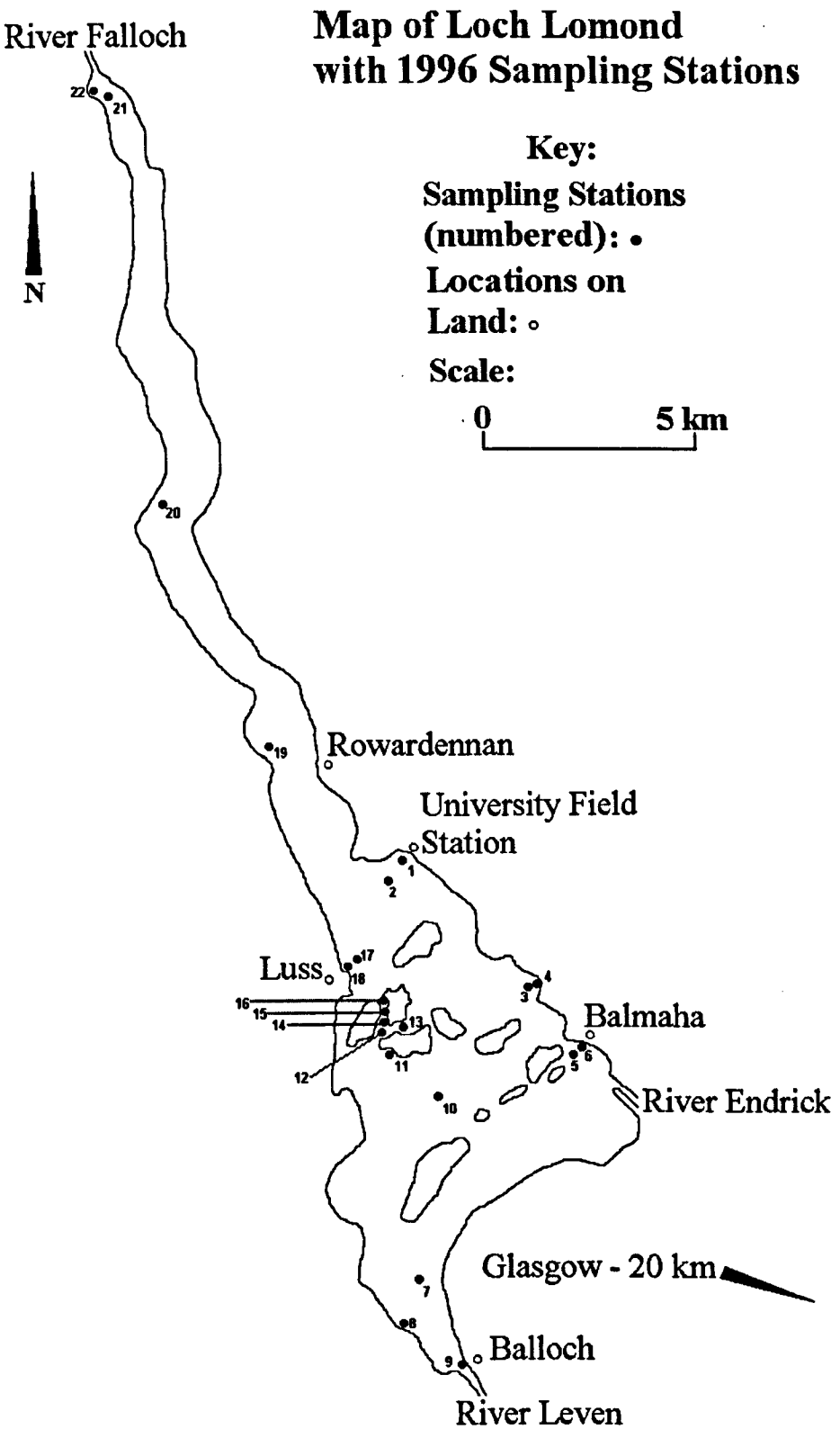

Fig. 5.2: Map of Loch Lomond, showing the sampling sites named and numbered as in Table 5.1.

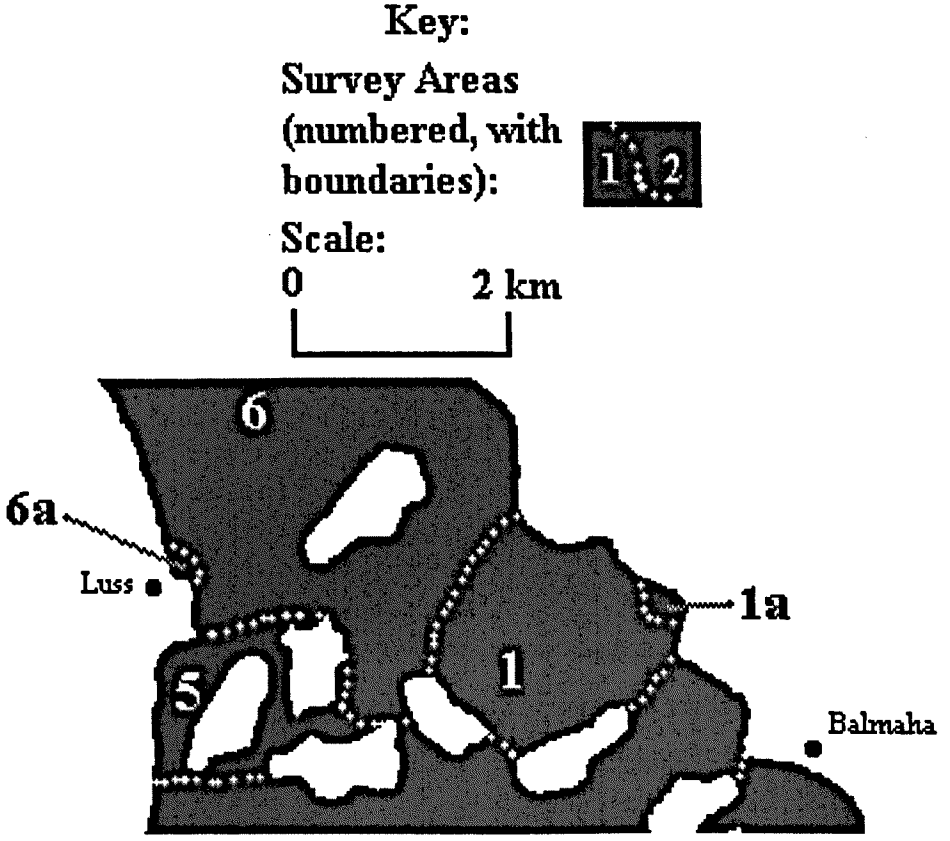

Fig. 5.3: *Map of a section of Loch Lomond, showing the positions of the two new boat census survey areas.*

5.3.2.3: Consistency of Volatile Aromatic HC Measurements at Selected Sites, During Summer Weekends

In order to examine the consistency of surface water volatile aromatic HC concentrations, one 1 l surface water sample was taken from each of four locations (Site Nos. 2, 6, 10 and 16, see Fig. 5.2 and Table 5.1) on three weekend days in the summer of 1996 (giving twelve additional water samples), in addition to July 21:

1) Saturday July 27
2) Saturday August 3
3) Sunday August 4

The four sites were chosen on the basis of boat census data, to represent a range of levels of powerboat activity (see Table 5.1). As in the experiment described in Section 5.3.2.2, boat census surveys were also carried out on each of these four days. In all, these surveys yielded twelve additional data points (to that of July 21), each representing local powered boat density and volatile aromatic HC concentrations at the four different sites and three different days. The data from these three days was combined for analysis with all of the data from July 21 to see if local boat density could predict surface water HC concentrations, with data from several different days.

5.3.2.4: Depth Profile of Powerboat Exhaust Hydrocarbons

(a): Field Experiments

On each of two days (Sunday July 28 and Sunday August 11 1996), water samples were taken from a range of different depths (surface, 25, 50, 75, 100 and 150 cm) at site No. 16 (see Table 5.1) at 5.00 p.m., just after the boat census was completed and just after the time of peak boat activity on that day (see Bannan *et al.*, 1995). This site is located in boat census survey Area 5, which had hitherto been consistently subjected to some of the highest levels of boat activity on Loch Lomond (see Adams and Brown, 1996). In the preliminary survey (see Section 3.3.3), powerboat exhaust HCs were detected in the surface waters of this site. Sub-surface samples were taken according to the method outlined in section 5.3.1.3.

(b): Controlled Experiments

Three controlled experiments investigating the depth profile of volatile aromatic HCs from powerboat exhaust were also performed. In all of these experiments, a "Mariner" 10 horsepower two-stroke outboard motor, running on a 50:1 mixture of

"Thames" leaded petrol and "Castrol 2-stroke Motorcycle Oil" was used to produce Exhaust-Polluted Water (EPW) in a 1787 litre stainless steel tank. In each experiment, the engine was run in this tank, filled with water taken from the Field Station's experimental supply, which is extracted from a depth of 2.5 m in the Field Station Bay of Loch Lomond (Ordnance Survey Grid Reference NS 377 957). The engine was run at 40 % throttle (between 2420-2850 rpm), for 10 minutes in each experiment.

All contaminated water samples (which were of 125 ml volume) were taken 1 hour after the cessation of engine operation, in order to allow any short-term settlement of the tank water to take place, if it occurred. As the tank had a maximum depth of only 75 cm (much less than 1.5 metres, which was the greatest depth from which water samples were taken from Loch Lomond), water samples were taken from an appropriate range of shallower depths: 0 (surface), 5, 10, 25, 50 cm and bottom.

Surface water samples were taken using the procedure described in Ch. 3, Section 3.2.1.2, while samples from depths of 25 cm, 50 cm and bottom depth were taken using the procedure described in Section 5.3.1.3. Samples from depths of 5 cm and 10 cm could not be taken using either of these two methods and instead were taken by carefully lowering a closed 125 ml sample bottle by hand to the required depth, using a ruler. Once the mouth of the bottle reached that depth, the lid was carefully removed and the bottle allowed to fill with water.

Bottom water samples were also taken 3 days and 12 days after running the engine (in the case of the latter two experiments) for comparison with the corresponding surface samples, to see if any settlement occurred over longer time intervals.

In all of the controlled experiments described in the current chapter, the amount of fuel consumed by the engine was measured. This allowed the approximate assessment of boat engine HC contamination as the ratio of fuel consumed by the engine and the amount of dilution water in the tank in which the engine was operated. Such a non-

empirical technique facilitated the comparison of the results of different studies, including older work in which aqueous volatile aromatic HC concentrations were not measured by any other more accurate means.

5.3.2.5: Recovery of Water Quality

(a): Following Peak Weekend Boat Activity (Field Experiments)

To investigate the potential for weekday recovery of water quality after summer weekend powerboat activity, 1 l surface water samples were taken at 5 p.m. on a Sunday afternoon (just following peak Sunday boat activity) and at regular time intervals after this, during the following week. This experiment was carried out twice, with water samples taken after 0, 14, 24, 48, and 72 hours in the first experiment (commencing on July 28 1996) and after 0, 14, 24, 48, and 60 hours in the second (commencing on August 11 1996), following peak Sunday afternoon boat activity. The last sample in the second experiment was taken on Wednesday morning at 6.00 am on August 14th 1996. This time was deliberately chosen as being before any significant boat activity that day and it was hoped that recovery of water quality could occur by then.

(b): Following a Simulated Pollution Event (Controlled Experiments)

To investigate recovery of water quality, a series of three controlled experiments were carried out. In these experiments, polluted water was produced by running the engine for 10 minutes in the 1787 litre stainless steel tank, as described in section 5.3.2.4(b). Immediately following termination of engine operation, a 125 ml sample of surface water was taken, using standard procedures (see Chapter 3 section 3.2.1.2). This sample was taken from a specified location in the tank, 10 cm away from the engine (in a direction towards the centre of the tank). Apart from controls, this was the first sample

taken in each experiment, corresponding to no time elapsed since the cessation of engine operation. Further samples were then taken in exactly the same manner, at time intervals approximating a logarithmic scale (as close to this as was practicable): after 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 24 hr, 48 hr and 72 hr, as a previous study (Boating Industry Associations, 1975) had shown a logarithmic relationship between time elapsed and the concentrations of powerboat exhaust pollutant concentrations in water, in controlled experiments. Extra samples were taken after 12 days (288 hours) in the latter two experiments.

To gain the best indication of the rate of decline, the half-life of the decline of volatile aromatic HC concentrations was calculated using regression analysis. This technique has often been routinely used in analysis of radioactivity (see Burchan and Jobes, 1995).

5.3.2.6: Rate of Introduction of Hydrocarbons to Freshwater

(a):Short-term

The rate of introduction of hydrocarbons into freshwater during outboard motor operation was investigated by running the "Mariner" engine in the 1787 litre tank for 10 minutes as described in Section 5.3.2.4(b). Surface water samples of 125 ml volume were taken (as described in Section 3.2.1.2) at the following times during engine operation:

- 5 seconds after commencement of engine operation.
- 30 seconds " " " " " .
- 2 minutes " " " " " .
- 5 min " " " " " .
- 10 min " " " " " (end of engine operation).

(b):Longer-term

A longer term experiment was also carried out. This was done by running a "Yamaha" 10 hp outboard motor in the 1787 litre stainless steel tank for a period of 3 hours 30 minutes at 1800 - 2000 rpm, measured by a "Revco REV 1" digital engine revolution counter (accurate to ± 0.2 %, from 100 to 19900 rpm). 125 ml water samples were taken, using the same procedure described in Section 3.2.1.2, at the aforementioned time intervals, up to 10 minutes following commencement of engine operation. In addition, samples were also taken at the following times after commencement of engine operation:

- 20 min.
- 40 min.
- 1 hour 45 min.
- 3 hours 30 min. (end of engine operation in this experiment)

The last sample was taken after a time period of engine operation, known to be appropriate to produce heavily polluted water, from earlier pilot experiments, described in Ch. 3, section 3.2.2). The resultant ratio of fuel consumed to the initial amount of water (prior to commencing experiments) in the tank was identical in both experiments

5.4: Results

5.4.1: Validation of the Field Water Sampling Procedure Used in the Current Study

The field sampling procedure in the current study was found to be acceptable, with very little risk of contamination of water samples from the sampling motor boat itself. Of the samples taken from various locations around this boat (see section 5.3.2.1) while the engine was running, the only one that contained detectable levels of hydrocarbons was that taken from directly behind the exhaust outlet, with a quantifiable aqueous concentration of the HC standard compounds of 1.58 $\mu g.l^{-1}$. This consisted of 1.12 $\mu g.l^{-1}$ of 1,3 dimethyl-benzene and 0.46 $\mu g.l^{-1}$ of 1,2 dimethyl-benzene. Unfortunately, toluene could not be quantified in this particular experiment, as it was present in all the samples, including the pure DCM. This could have emanated from atmospheric contamination of the laboratory with toluene, which is a standard solvent, often used and kept in considerable quantity in the Organic Chemistry Laboratory at SEPA West, East Kilbride.

As expected, no moving boats other than the sampling vessel were observed throughout the course of this experiment.

5.4.2: Field Experiments - General

Concentrations of volatile aromatic HCs of over 30 $\mu g.l^{-1}$ were detected in four of the 78 (mostly surface water) samples taken during the summer of 1996. Lower, but still elevated concentrations of above 4 $\mu g.l^{-1}$ were detected in 38 samples. The six samples with the lowest concentrations had levels of less than 1 $\mu g.l^{-1}$, which is not far above detection limits (0.4 $\mu g.l^{-1}$, see Ch. 4, Section 4.3.7(a).2. Of the 25 samples taken from open water sites of low boat activity, only one had levels of over 4 $\mu g.l^{-1}$. All 9 of the

reference samples taken from a depth of 1.5 m at Site No. 2 (see Section 5.3.2.2(a)) contained even lower levels of volatile aromatic HCs, with a mean concentration of only 1.47 $\mu g.l^{-1}$ (S.E. 0.19 $\mu g.l^{-1}$; see Table 5.3).

Table 5.3: Concentrations ($\mu g.l^{-1}$)of volatile aromatic HCs in water from a depth of 1.5 m at Site No. 2 on nine occasions during the summer of 1996.

Date	*Concentration ($\mu g.l^{-1}$)*
21.7.96	2.70
27.7.96	0.78
28.7.96	1.05
31.7.96	1.76
4.8.96	0.91
11.8.96	1.54
12.8.96	1.54
13.8.96	1.31
14.8.96	1.63

5.4.3: Spatial Distribution of Powerboat Exhaust HCs in Loch Lomond

On the 21st of July 1996, concentrations of volatile aromatic HCs in surface waters ranged from a relatively low level of 2 $\mu g.l^{-1}$ at Site 5 to 34 $\mu g.l^{-1}$ at Site 4. The concentrations of volatile aromatics at each site are shown in Fig. 5.4, with the sites numbered as indicated in the scheme in Table 5.1. Using the data from this experiment alone, local boat density (in each boat census survey area) was a reasonably accurate predictor of surface water volatile aromatic HC concentrations. Using regression analysis, boat density was found to positively correlate with HC concentrations, yielding a strong statistical relationship ($F_{1,10} = 76.16$, $r^2 = 88.2$ %, $p < 0.001$; regression analysis). Fig 5.5 graphically depicts this statistical relationship.

Fig. 5.4 also gives an indication of sampling variability. This was gained from the S.E. of the mean volatile aromatic HC concentrations within each of the three designated groups, with "low", "moderate" and "high" powerboat activity (as designated in Sec 5.3.2.2(b)). For each sample, the error bar represents the S.E. of the volatile aromatic HC

concentration of the particular category of powerboat activity to which the sample belongs. Table 5.4 presents descriptive data of boat density and mean HC concentration within each of the three designated powerboat activity groups.

Table 5.4: Boat numbers and volatile aromatic HC concentrations in the three designated categories of powerboat activity on July 21 1996.

Designation	n	Mean (active powerboat numbers)	Range (active powerboat numbers)	Mean (volatile aromatic HC concs.)	Median (volatile aromatic HC concs.)	S.E. (volatile aromatic HC concs.)	Range (volatile aromatic HC concs.)
Low	9	4.2	1.2–5.6	5.0	4.4	0.9	1.7-10.3
Moderate	6	9.7	7.0-11.6	8.3	6.6	2.0	5.5-18.3
High	7	114.6	43.1-360.0	15.9	10.9	4.7	4.8-33.6

It was also found that the there was a statistically significant difference between the medians of the three groups, with respect to their volatile aromatic HC concentrations ($H = 7.73$ d.f. $= 2$ $p = 0.021$; Kruskal Wallis one-way ANOVA).

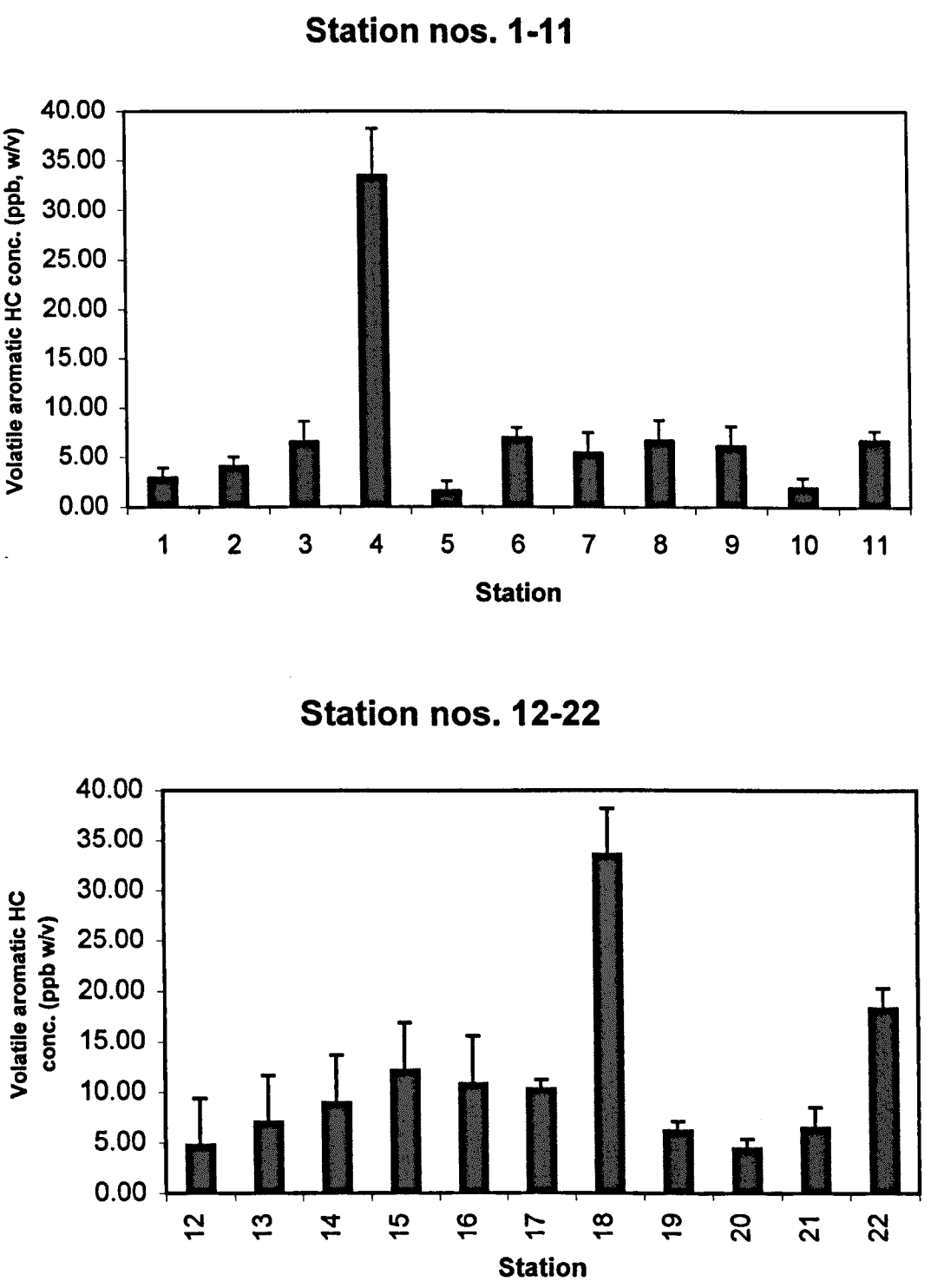

Fig. 5.4: Concentrations of volatile aromatic HCs in surface water samples taken on July 21st, 1996 at 22 sites in Loch Lomond. The sites are numbered according to the scheme in Table 5.1 and also indicated on the map in Fig. 5.2. An indication of sampling variability is also given, as described in Section 5.3.2.2(b)

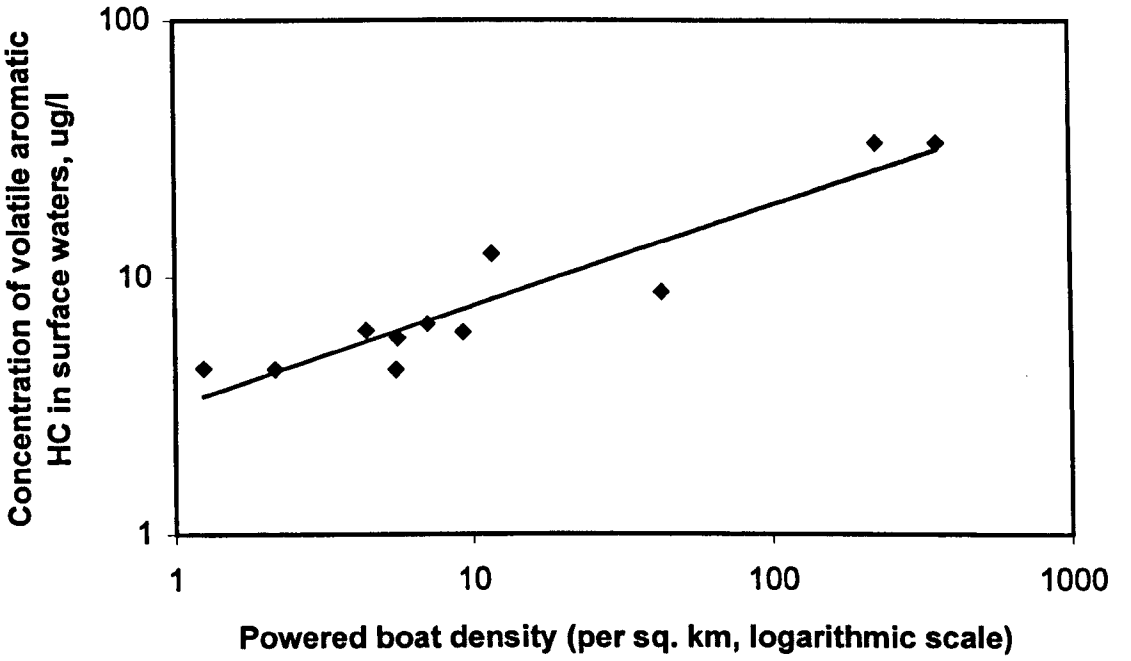

Fig. 5.5: Local powered boat densities in 11 boat census survey areas, plotted against volatile aromatic HC concentrations in the surface waters of each of these areas of Loch Lomond, on July 21 1996. Both axes are on a log_{10} scale.

5.4.4: Variation of Hydrocarbon Concentrations

When the data from all of the weekend days is combined (as described in Section 5.3.2.3), a much weaker statistical relationship between local boat densities and surface water HC concentrations is gained (regression analysis: $F_{1,22} = 9.73$, $p < 0.005$, $r^2 = 27.5$ %) than from that using data from July 21 only. The combined data is displayed in Fig 5.6.

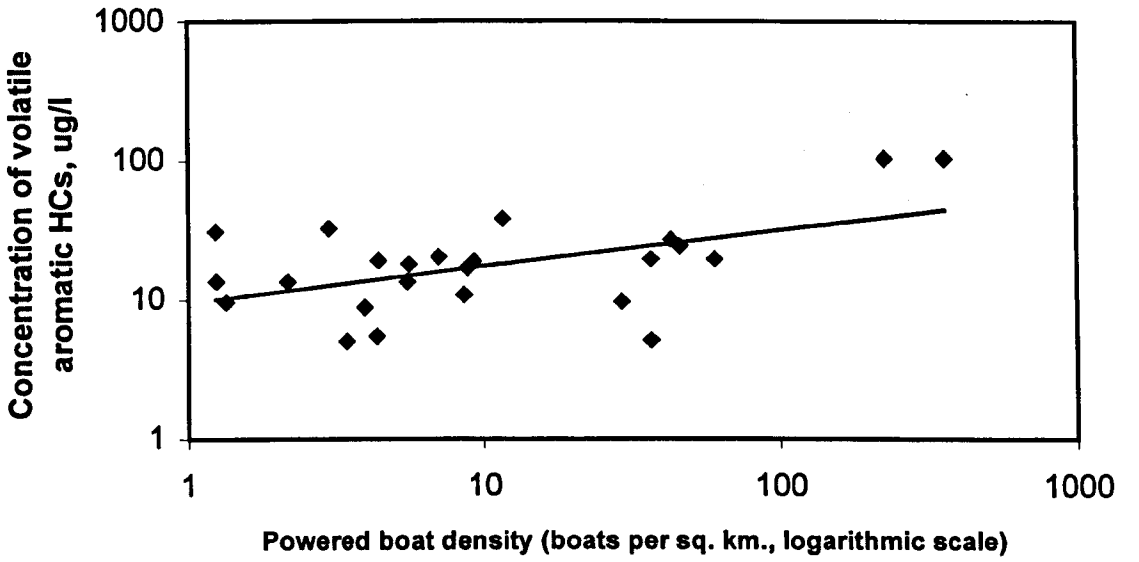

Fig. 5.6: Local powered boat densities plotted against volatile aromatic HC concentrations in the surface waters of Loch Lomond, with data from four summer weekend days in 1996; both axes are on a log_{10} scale.

5.4.5: Depth Profile of Powerboat Exhaust Pollutants

5.4.5.1: Field Experiments

Fig. 5.7 shows the concentrations of volatile aromatic HCs at depths ranging from surface to 1.5 m at site No. 16 on Sunday July 28, 1996 (A) and Sunday August 11, 1996 (B). As can be seen from the data from both experiments, it is apparent that there was no decrease in HC concentration with depth. Indeed, in the first experiment, an apparent increase with depth occurred. Unfortunately, one of the sampling devices was smashed before taking samples on August 11, 1996 and no sample from 75 cm depth was taken on that occasion.

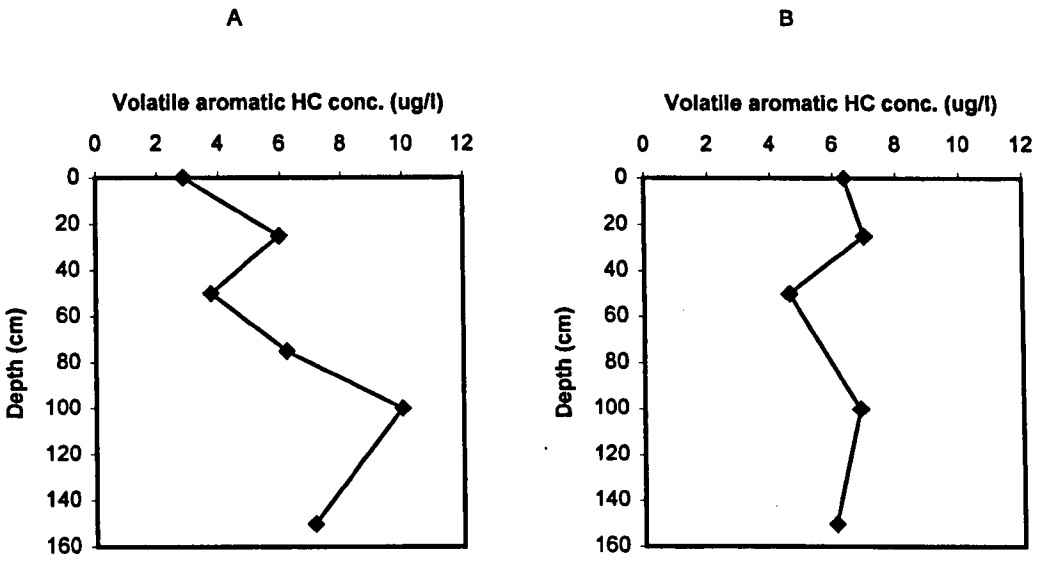

Fig. 5.7: Depth profile of volatile aromatic HC concentrations at the Narrows on two occasions - (A) Sunday July 28, 1996 and (B) Sunday August 11, 1996.

5.4.5.2: Controlled Experiments

The results of the three controlled experiments, in which the depth profile of volatile aromatic HCs in the tank was investigated, show that there was no statistically significant trend of HC concentration with depth ($F_{2,17}$ = 0.48; n.s. ; one way ANOVA). Fig 5.8 represents the volatile aromatic HC concentrations with depth in the tank in these three experiments.

The total depth of water in the tank, after the completion of engine operation was 62, 66 and 66 cm in each of the three experiments respectively, as engine operation caused some spillage. On examination of surface and bottom water samples taken 3 days following termination of engine operation, it was found that there was no statistically significant difference between surface and bottom water HC concentrations (t = -1.86, p = 0.16, 3 d.f.; paired t test). There were also no great differences after 12 days. In the two experiments that were prolonged for this time, the mean surface water concentration was

3.14 mg.l^{-1} and the mean bottom water concentration was 3.57 mg.l^{-1}.

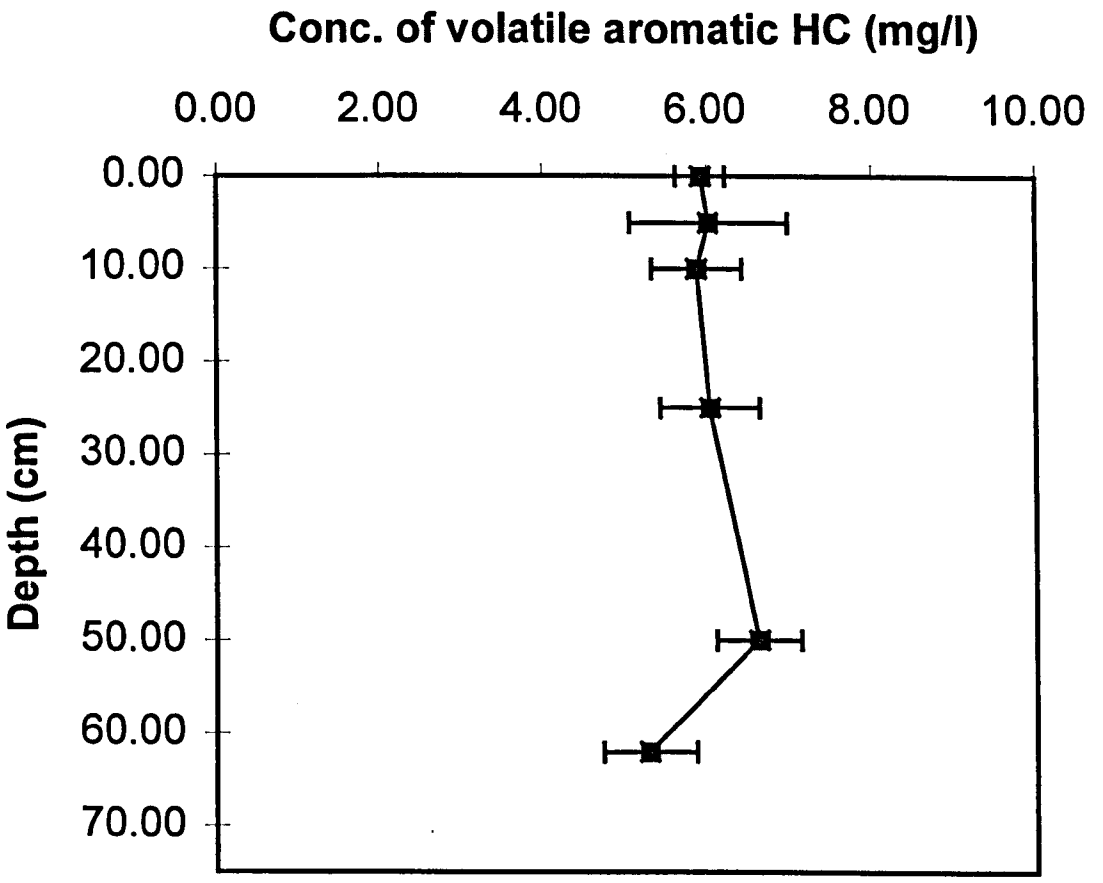

Fig. 5.8: The depth profile of volatile aromatic HCs in EPW in three controlled experiments: mean values with standard error bars are displayed.

5.4.6: Recovery of Water Quality from Powerboat Exhaust HC Contamination

5.4.6.1: Field Experiments

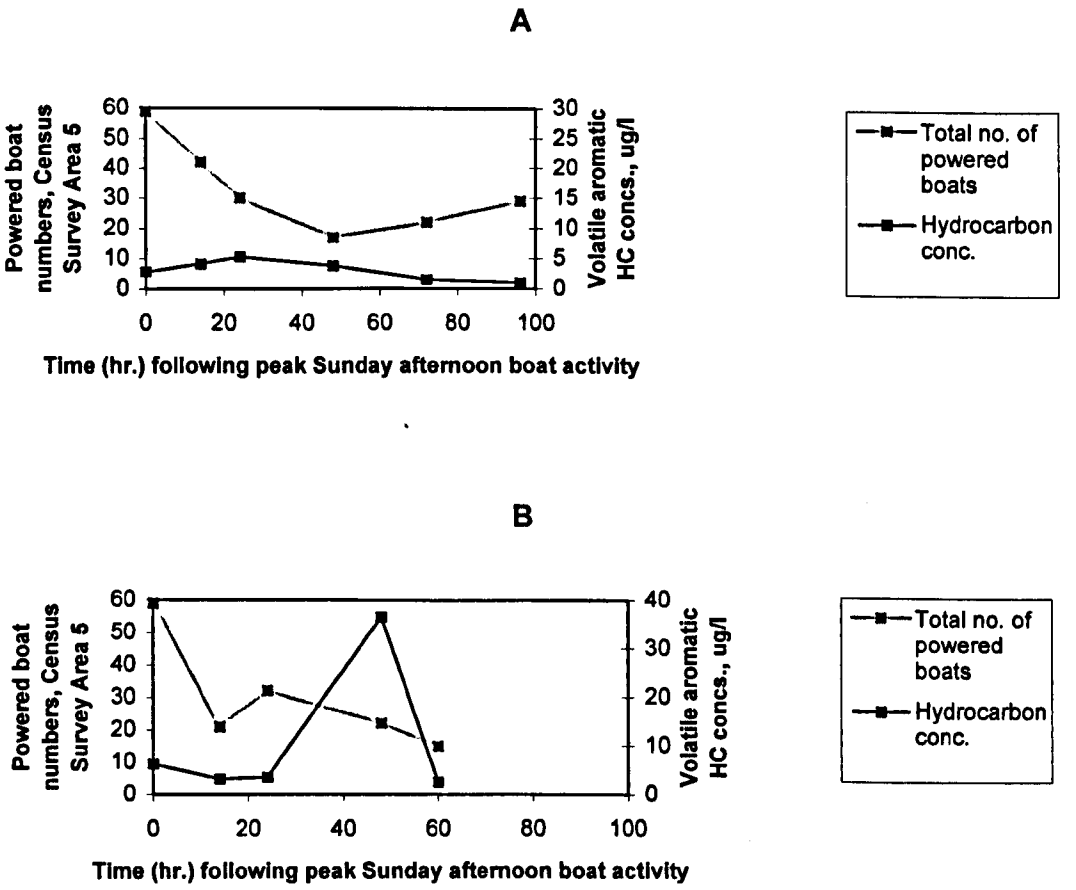

Fig. 5.9: Surface water total volatile aromatic HC concentrations at Site 16 and powered boat numbers in boat census survey area 5, following peak Sunday powerboating activity in two experiments, carried out in the summer of 1996:
A) from July 28 to 31
B) from August 11 to 14

Figs. 5.9 (A) and (B) show the trends in the numbers of powered boats (counted as in use on a particular day – see Ch. 2, Section 2.2.1.1) and surface water volatile aromatic HC concentrations from Sunday July 28 to Wednesday July 31, 1996 and from Sunday August 11 to Wednesday August 14 1996 respectively, measured at Site 16 in survey Area 5 of Loch Lomond. No clear trend in HC concentration with time is evident in either experiment, despite the big drop in the numbers of active powered boats on weekdays. In the second experiment, there was a large increase in the total concentrations of surface water volatile aromatic HCs at a time with very low powerboat activity.

5.4.6.2: Recovery of Water Quality, Controlled Experiments

The changes in volatile aromatic HC concentrations with time in the three controlled experiments, carried out in the 1787 litre stainless steel tank, are shown in Fig. 5.10. It was found that a gradual, if slightly erratic decline occurred in each of these experiments. Unfortunately, it was not possible to calculate a half-life for the decline of volatile aromatic HCs from the combined data from all three of the experiments. Although the slopes of the three individual regression lines (representing time versus volatile HC concentration in each of the experiments) are not significantly different ($F_{2,24}$ = 3.27, n.s.), indicating similar rates of decline of HC concentration, the elevations are ($F_{2,28}$ = 4.41, p = 0.022), indicating differences in the initial HC loading of the water in each experiment. These differences produced significantly different data sets, which cannot be meaningfully combined for analysis. It was thus necessary to calculate half-lives for each of the three experiments separately and calculate the mean of the half-lives. The results of the regression analyses of each of the three individual experiments are shown in Table 5.5, in addition to the half-life calculations for each. For all of these analyses, time (measured in hours) was $\log_{10}$ transformed. Two estimates of the half-life are given:

- the time elapsed from the conclusion of engine operation to half the measured concentration at that time.

- the time elapsed from the conclusion of engine operation to half the nominal initial concentration estimated by regression analysis, i.e. the intercept of the regression line.

The mean of the three half-life calculations was found to be 155.9 hours or just under 6.5 days using measured initial concentrations (see column 7 in Table 5.5) or 208.9 hours or 8.7 days (using nominal initial concentrations, gained from the intercept of the regression line with the y axis - see column 8 in Table 5.5).

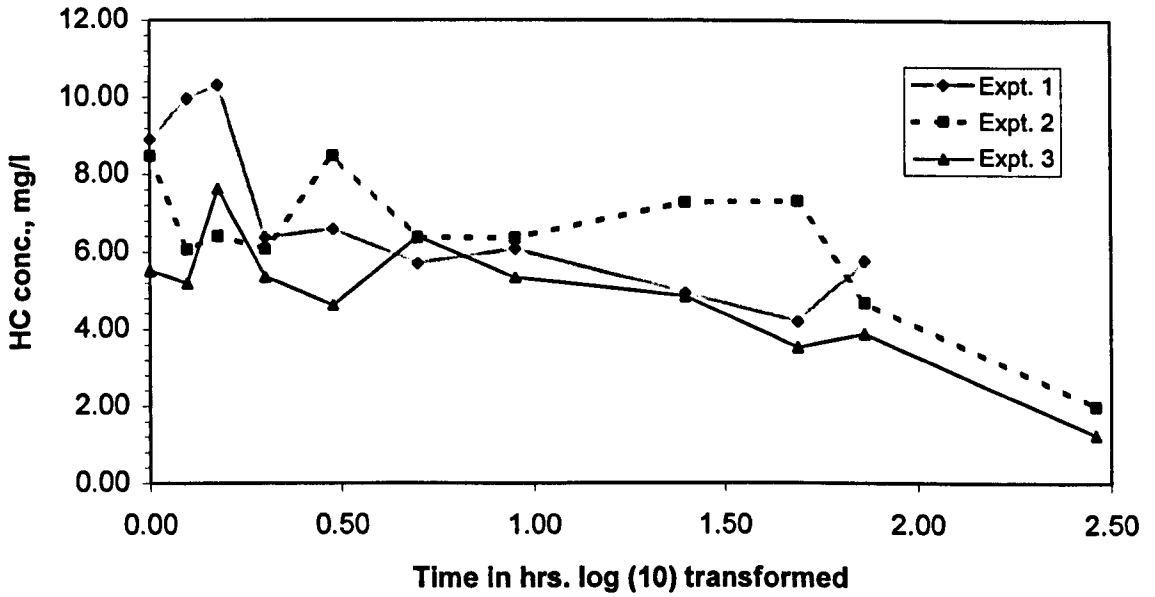

Fig. 5.10: Trends in aqueous volatile aromatic HC concentrations following a simulated pollution event in three controlled experiments, with time measured on a log_{10} scale.

Table 5.5: Results of regression analyses of the trends in aqueous volatile aromatic HC concentrations with time (log_{10} transformed for analysis) in each of three experiments in the present study. Half-lives for HC depreciation (not log transformed) are shown in columns 7 and 8. Half-life 1 is that calculated using measured initial concentrations while half-life 2 is calculated using nominal initial concentrations, calculated using the regression equations (i.e. the intercept of each regression line with the y axis).

Experiment	Duration	No. of samples	F	r^2	p	Half-life 1 (hr)	Half-life 2 (hr)
1	3 days	10	14.08	59.2 %	0.006	55.0	58.5
2	12 days	11	6.51	35.5 %	0.031	239.8	475.7
3	12 days	11	17.52	62.3 %	0.002	172.9	92.4

5.4.7: Rate of Entry of Exhaust Pollutants to the Water

Fig. 5.11 shows the trend in aqueous volatile aromatic HC concentrations during engine operation in three controlled experiments. HC input appeared to be directly proportional to the time of engine operation for the first 10 minutes. The data from the three engine trials was combined for regression analysis, first by expressing each HC measurement as a proportion of the final concentration, measured after 10 minutes in each experiment. The data was then arcsine transformed before a preliminary ANCOVA analysis, in which it was found that there was no significant differences in slope ($F_{2,12}$ = 0.08, n.s.) or elevation ($F_{2,14}$ = 3.51, n.s.) of the individual regression lines. This allowed the three data sets to be combined for analysis. Regression analysis of the combined data showed that there was a highly statistically significant relationship between the time of engine operation and the concentration of volatile aromatic HCs in the water ($F_{1,17}$ = 410.57, $p < 0.001$, r^2 = 96.0 %, 17 d.f.). The rate of input can then be calculated and this was found to be 1.37 g of volatile aromatic HCs per minute of engine operation.

In the longer-term experiment, in which an engine was run in the 1787 litre tank for 3 hr 30 min, the rate of hydrocarbon input was found to progressively slow down after 10 minutes of engine operation and the relationship between time and HC concentration becomes non-linear (see Fig. 5.12).

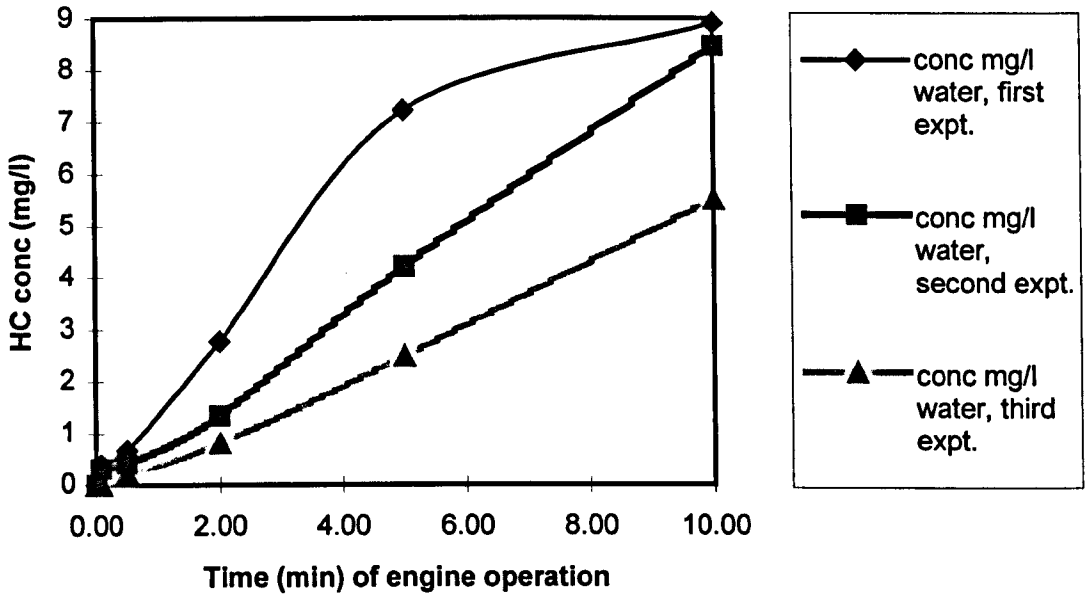

Fig. 5.11: The trend in aqueous concentrations of volatile aromatic HCs during three experiments, in each of which an engine was run in a 1787 l tank for 10 min.

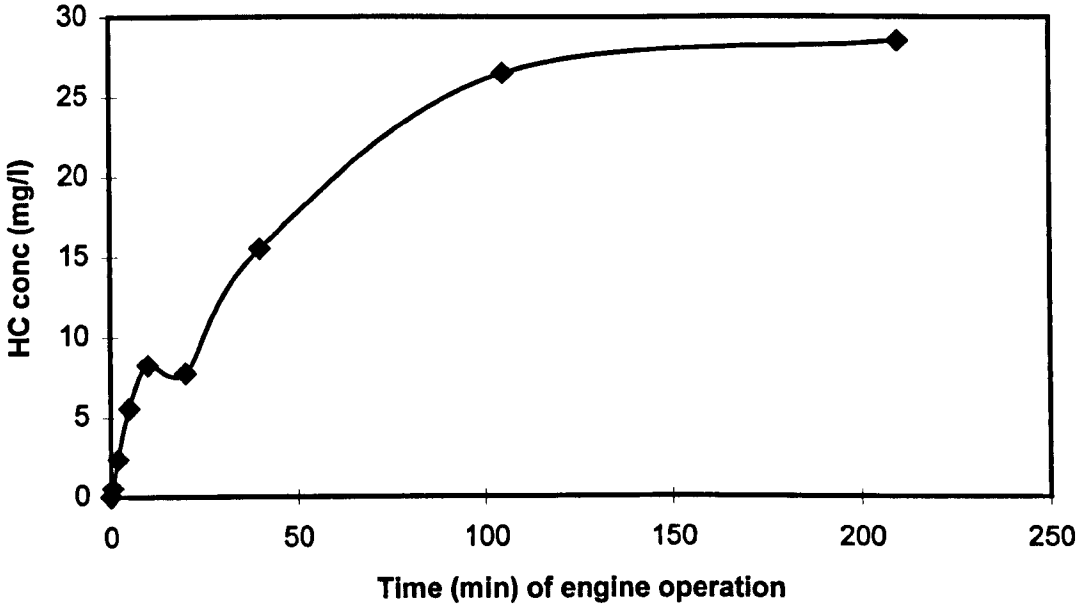

Fig. 5.12: The trend in aqueous concentrations of volatile aromatic HCs during an experiment in which an engine was run in a 1787 l tank for 3 hr 30 min.

5.5: Discussion

5.5.1: General: Main Finding and Verification of Sampling Procedure

The results of this study show unequivocally that petroleum HC contamination from powerboats occurs during summer, in areas of high boat activity in Loch Lomond. This contrasts with the conclusions of some previous studies (Boating Industry Associations, 1975; Wachs *et al.*, 1992). In both of those studies, it was concluded that heavy powerboat activity did not significantly alter aqueous HC concentrations, even in artificial lakes with much higher HC loading than that expected to occur in the field.

Levels of volatile aromatic HCs of up to 34 $\mu g.l^{-1}$ were found in the surface waters of Loch Lomond in the survey of July 21 1996. These levels are very comparable to the concentrations of a very similar range of substances (viz: Benzene, Methyl-benzene (Toluene), Dimethyl-benzenes (Xylenes), Ethyl-benzene and Methyl-tert-Butyl Ether (MTBE)) detected in Lake Tahoe, California, USA by Miller and Fiore (1997). In that study, levels of these compounds of up to 47 $\mu g.l^{-1}$ were measured. Between them, those compounds would be expected to comprise over 78 % of the total amount of volatile aromatic HCs in petrol (Johansen *et al.*, 1983).

In other previous studies (Mantoura *et al.*, 1982), similar compounds were measured in coastal seawater at Vineyard Sound, Massachusetts, USA, but the levels were found to be much lower, with a maximum of fifty times less than that found by Miller and Fiore (1997). This could have been due to the much higher degree of dispersal and mixing that would occur at a marine coastal site, compared to a relatively small water body, such as Loch Lomond or Lake Tahoe. Another possible contributing factor is the lower solubility of HCs in seawater compared to freshwater.

The results presented in this chapter effectively rule out atmospheric deposition and road runoff as major sources of HCs in the waters of Loch Lomond, confirming the tentative conclusions stated in Ch. 3, Section 3.4.3. If atmospheric deposition did occur, the HCs from this source would be:

- expected to be predominantly PAHs.
- expected to have an even distribution throughout the surface waters of Loch Lomond.

In actual fact, the HCs actually found were mostly volatile aromatics and a strong positive correlation was found between local powerboat densities and aqueous volatile aromatic HC concentrations in surface waters. This indicates that powerboats were the chief source of aqueous HC contaminants in Loch Lomond.

Road runoff is also unlikely, as in addition to different HC compounds present (aliphatic HCs and PAHs), road runoff would be expected to occur mostly near areas of drainage from roads. This was not found to be the case, and most of the samples with the highest aqueous HC concentrations were found at or near Site No. 16, which is located in a channel between two islands and at least 1.5 km from the nearest public road, which itself is on the mainland. It would thus seem that the likelihood of road-derived HC contamination reaching Site 16 (and many of the other sites) would be minimal.

5.5.2: Spatial Distribution of Powerboat Exhaust Hydrocarbons in Loch Lomond

In the survey carried out on July 21 1996, local boat densities were highly correlated with the concentrations of powerboat exhaust HCs in surface waters. The highest HC concentrations were all found either in areas with correspondingly high boating activity (at Sites Nos. 3,4,15 and 16) or high refuelling activity, where fuel spillage can occur (Site No. 18). Although fuel spillage is most likely at times of high powerboat activity (as witnessed on July 21 1996), it can occur at unexpected times. This happened at Site No. 16, from 5.00 p.m. on Tuesday August 13 to 6.00 a.m. on

Wednesday August 14, 1996, where despite low craft densities, a high HC concentration was detected. This area is often used as an overnight stopover point for many boats and boat refuelling may take place here. It is suspected that the relatively high concentrations of HCs recorded at this time may be the result of spillage. Further research in this subject could be useful, in order to more thoroughly investigate the potential for such episodes of contamination in Loch Lomond.

There could also be a significant number of old, inefficient and highly polluting engines still operating on Loch Lomond. Contamination from such an engine may have occurred at site No. 18 on July 21 1996. Like all of the sites, this site was pre-selected, but on the day of sampling, a large tour boat was operating and visibly discharging unburned fuel. Oily slicks were observed, and the HCs extracted from the surface water sample yielded fluorescence spectra indicative of diesel fuel (see Appendix VII).

Almost all of the open water sites with low boating activity contained correspondingly low levels of volatile aromatic HCs. Elevated concentrations only occurred on one occasion, reaching 4.42 $\mu g.l^{-1}$ at site No. 20 on July 21, 1996. In addition, the reference samples taken on nine occasions from a depth of 1.5 m from site 2 all had very low concentrations of HCs.

There was some degree of scatter in the regression plot of the relationship between local boat densities and the concentrations of surface water volatile aromatic HCs on July 21, 1996, demonstrated in Fig. 5.5. This variation could have been due to the patchy nature of HC pollution, which has previously been demonstrated in the case of marine oil pollution (see NRC, 1985). It could be that although the large scale and long-term distribution of boats has been shown to be predictable (see Adams *et al.*, 1992; Adams and Grant, 1997), the localised (at the smallest scale) and short-term distribution of boats could be highly erratic, leading to a correspondingly erratic distribution of surface water powerboat exhaust HCs.

It may be possible to find a better relationship between boat densities and aqueous volatile HC concentrations by counting boats in smaller areas, but this would be require a more intensive study. A more practical alternative approach could be to take many surface water samples from each census survey area, to get some indication of the "average" sample within each of the 14 boat census survey areas.

A better method of surface water sampling could also prevent unnecessary variation. In the current study, the method for surface water sampling was quick, and simple, with very little risk of sample contamination (see Ch. 3, Section 3.2.1.2), but could be imprecise. Although it is likely that no water deeper than 2 cm was taken, this is not certain. In a previous study (Gordon and Keizer, 1974) surface water sampling devices were used, and their correct deployment described. It may be useful to use similar devices in future work on powerboat exhaust contamination of Loch Lomond. An alternative approach could investigate the effect of powerboat activity on sub-surface water. This would benefit from initial experiments to investigate the variation of sub-surface water HC concentrations at one site, using the existing methods, outlined in section 5.3.1.3.

It is also possible that different weather conditions could have an effect on the distribution of surface water HCs in Loch Lomond. Previous research has found that wind can be a major factor in determining the distribution of surface water HCs (Pople *et al.*, 1990; Wachs *et al.*, 1992) and different weather conditions (particularly wind speed and direction) could have helped to transport HCs within the loch. Fig 1.1, Ch. 1 gives a breakdown of the prevailing wind direction at a nearby site (Glasgow Airport, less than 30 km from the southernmost reaches of the loch) and although the most frequent prevailing wind is south-westerly, there is a great heterogeneity of wind direction throughout the year.

Currently, not a lot is known about water circulation patterns in Loch Lomond, with one exception being a study by Curran and Poodle (1994). In that study, it was found that dilution rates could be quite rapid in open water. More background work in this area is vital before any future work about the environmental fate of HCs in Loch Lomond, involving both controlled and field experiments. It may be useful to measure dilution rates in enclosed water sites, where most boating activity occurs.

Additional variation in the concentrations of volatile aromatic HCs may be caused by the wide variety of engine sizes and types present in Loch Lomond, all having different emission rates and using different fuels. Further research, involving both censuses of the numbers of boat engines over a certain age, and also the extent of fuel spillage (perhaps using a mathematical modelling approach) from all vessels would be useful to complement the present study.

Only a very weak statistical relationship between local boat densities and surface water HCs resulted when the data from all four weekend days was combined for analysis, in contrast to that obtained from the data collected on July 21 alone. Also, in the case of the combined data, the actual differences in weather conditions between different days constituted an extra variable (which could have had a profound influence), not present in the one-day survey of July 21, 1996. Another likely explanation of the variation in surface water HC concentrations between different days is the real differences in boat numbers and types between Saturdays, Sundays and different holiday types. Such differences in boat distribution patterns have already been described for Loch Lomond in Brown and Adams (1995).

5.5.3: Depth Profile of Powerboat Hydrocarbon Pollution

The results of both the Loch Lomond survey and the controlled experiments in the current study showed that contamination of water from powerboat exhaust is not confined to the surface microlayer (the top 60 μm), but is found to depths of at least 1.5 m. The results of the only comparable previous study (Miller and Fiore, 1997) confirm this. In that study, volatile aromatic HCs and MTBE (methyl tert-butyl ether) were found at depths of at least 4 m in Lake Tahoe, California, USA. This high degree of vertical mixing contrasts with the behaviour of some other types of organic compounds, which have much lower aqueous solubility (see Table 5.6). Hardy *et al.* (1990) measured the concentrations of some of these other organic compounds (including polycyclic aromatic hydrocarbons, PAHs) in the water column of Chesapeake Bay, Maryland, USA. In that study, it was found that these particular organic compounds were almost completely confined to the surface microlayer.

It must be borne in mind that a small amount of surface water contamination may have occurred in the method used for sampling sub-surface water. As the device was lowered, a small column of surface water, including a small area (approximately 0.5 cm^2) of the surface film entered the mouth of the separating funnel. When the sample was taken, this small amount of surface water would have been sucked into the sampling vessel and could have caused contamination. However, the amount of surface film would have been very small compared to the corresponding area taken in surface water samples (possibly one thousand times less) and any contamination from the surface film is very unlikely to explain the almost uniform vertical distribution of volatile aromatic HCs found both at Site 16 in Loch Lomond and in controlled (tank) experiments.

No significant settlement (either towards the surface or the bottom waters of the tank) took place within the full twelve day time period of the controlled tank experiments. This suggests that despite the low density of HC compounds from outboard motor exhaust, they do not float at the surface as slicks, but are dissolved in the water in

appreciable concentrations. Organic pollutants from powerboat exhaust consist mostly of volatile monocyclic aromatic HCs (Jüttner *et al.*, 1995*a*), which in addition to being highly volatile are also much more water soluble than any other class of HC compounds. Table 5.6 compares the solubility of several different HC compounds (of different classes; data from Peterson, 1994). However, more research is needed, particularly involving the production of depth profiles at various time intervals after engine operation, possibly using deeper experimental tanks or even mesocosms.

Table 5.6: The solubility of powerboat exhaust pollutant compounds compared to that of other HC compounds. Data from Shiu *et al.* (1988).

Compound	*Chemical Formula*	*Class of Compound*	*Water solubility (mg.l-1)*
Benzene	C_6H_6	Volatile aromatic	1780.00
Toluene	C_7H_8	Volatile aromatic	515.00
Naphthalene	$C_{10}H_8$	PAH	35.00
Methyl-Naphthalene	$C_{11}H_{10}$	Substituted PAH	8.36
n-hexane	C_6H_{14}	Aliphatic	13.00
n-heptane	C_7H_{18}	Aliphatic	3.00
Indane	C_9H_{10}	Napthenic (Cyclic Aliphatic)	0.10

In the current study, there may have been a possibility of a "wall effect". By this, greater concentrations of volatile aromatic HCs than expected could have been found in bottom water samples from the tank, as these samples were taken from adjacent to the tank wall. However, in the field experiments, no depth profile whatsoever manifested itself, up to a depth of 1.5 m. Since such a pattern also existed in the tank experiments, it can be concluded that such a "wall effect" was unlikely.

In the field, this high degree of solubility could enable these compounds to be mixed into deeper water by propeller agitation and water currents. More field experiments are needed, as the depth profile at only one site was investigated. This site was subjected to heavy powerboat activity, but was atypical of most of Loch Lomond, as it was located

in an enclosed channel between two islands. It could be useful to investigate HC depth profile in a more open site in the loch, which would involve the sampling of very deep water, of at least 20 metres. All classes of HCs could also be examined. Such experiments could be useful in the investigation of the environmental fate of HC compounds. This would require a different method of water sampling, possibly using modified (preferably PTFE lined) boat-operated sampling vessels.

The relatively high solubility of volatile aromatic HCs in powerboat exhaust, demonstrated in controlled and field experiments, indicates that there may be a greater potential for these compounds to have adverse effects on aquatic organisms than more insoluble compounds. All petroleum oils contain volatile aromatic HCs and a significant proportion of the aquatic toxicity of these oils comes from these compounds (John and Soutar, 1981; NRC, 1985).

There is some evidence that pollution by fuels other than petrol (particularly diesel) may be significant in Loch Lomond. Oily slicks (probably diesel - see Appendix VII for more details) were found on three occasions at two sites (Nos. 16 and 18) in Loch Lomond. Unfortunately, no sub-surface water samples were taken from underneath these slicks and further work is required in order to investigate the vertical distribution of all types of petroleum oils in freshwater. Previous work on marine oil spills has shown that diesel, like crude oil, is largely found in the form of surface slicks (see Connell and Miller, 1984; Pople *et al.*, 1990). This is not surprising, as both of these oils largely comprise compounds which are much less water soluble than the principal compounds in powerboat exhaust or neat petrol (Shiu *et al.*, 1988; NRC, 1985). However, previous research (Gaur and Singh, 1989) has shown that weight for weight, diesel may be as much as twice as toxic to some aquatic species than petrol. More research is needed, particularly comparing the toxicity of petrol-derived and diesel-derived exhaust-polluted water, including the investigation of the water-soluble components of diesel.

5.5.4: Recovery of Water Quality Following Powerboat Pollution

(i) Field Experiments

In Loch Lomond, recovery of water quality was difficult to demonstrate, as contamination by powerboat exhaust HCs was unpredictable. In the first experiment, the site studied (The Narrows, site no. 16) had relatively low surface water volatile aromatic HC levels (only 2.88 μgl^{-1}) on Sunday July 28 1996, despite comparatively high numbers of powerboats throughout the loch, including the area in which the sampling site was located. HC concentrations were considerably higher in sub-surface water on that day, and also higher in surface waters on subsequent days of the experiment, despite much lower boat numbers occurring on those days (which were weekdays). Future research, involving large-scale water sampling, is necessary to more accurately ascertain the small-scale distribution of these contaminants.

In the second experiment, higher levels of volatile aromatic HCs than in the first experiment were found in the surface waters of site No. 16 at the beginning of the experiment (5.00 p.m. on Sunday August 11, 1996). An apparent drop occurred in the first 14 hours, possibly indicating some recovery of water quality. However, the situation was complicated thereafter by the presence of a large oily slick (most likely to be diesel fuel – see Appendix VII), which occurred at a time when boat activity was very low, showing that HC contamination can occur at unexpected times. Although the site (No. 16) shows apparently rapid (within twelve hours) recovery, an extra sample taken from only 200 m away showed that the slick was still present nearby. It may have been transported by wind or water currents, as can happen at sea (see Pople *et al.*, 1990).

It was intended to take the final sample in the second field experiment after 72 hours (i.e. at a time of 5.00 p.m. on August 14, 1996), just like in the first. However, some moving powered craft were observed at all times in census survey area 5 on every occasion on which water samples were taken in the first experiment, even at 7.00 am on

July 29, 1996. It was consequently felt that the second survey would benefit from incorporating a sample taken at a time at which clearly no moving boats were observed. The best time for this was decided to be 5.00 am in the morning on August 14, 1996, which also had the benefit of a period of at least 10 hours of negligible boat activity prior to sampling. Previous research (Mele, 1993; Bannan *et al.*, 1995) has shown that boat activity is mainly confined to a three to five hour period in the afternoon. Further research is necessary to investigate longer-term recovery of water quality, throughout the course of an entire calendar year.

(ii) Controlled Experiments

In a pilot experiment (carried out in a 510 litre tank; not described here), recovery of water quality was apparently fairly rapid following an artificial outboard motor pollution event. It was thus thought to be unnecessary to prolong the subsequent three experiments (carried out in the 1787 litre stainless steel tank) beyond the duration of 3 days. However, preliminary data analysis of the first of these experiments showed that recovery was slower than expected, with a mean of 64.60 % of the initial HC concentrations remaining after 3 days. In the second and third experiments, an extra sample was taken after 12 days and even then, a total of 23.45 % and 22.86 % respectively, of the initial HC concentrations remained in the two experiments respectively. This was much higher than expected from the pilot experiment, in which the 12-day total was probably less than 5 % of the initial amount (based on approximate quantities from a preliminary GC-MS analysis).

The later experiments were carried out in a more carefully controlled manner than the pilot experiment and there are a number of possible reasons for the apparently rapid recovery of water quality in the latter:

a) The smaller capacity of the tank used in the pilot experiments could have hastened the decline in aqueous HC concentrations. It is possible that HC evaporation rates are

higher for smaller water bodies.

b) There was a far higher initial pollution loading of the water in the pilot experiment. Engine operation significantly warmed the water (unlike in later experiments) and this could have facilitated a greater rate of HC evaporation.

c) Only three samples were taken in the pilot experiment. No indication of the heterogeneity of the water in the tank could have been gained from such a small number of samples and thus, the real pattern of decline could not have been properly ascertained. In the later experiments, the decline in HC concentrations was found to follow an erratic pattern, possibly due to such heterogeneity, even in a relatively small water body.

Although the decline in HC concentrations was found to be erratic in the controlled experiments, a logarithmic relationship between time elapsed and aqueous volatile aromatic HC concentrations could clearly be seen in each experiment, justifying the decision to take water samples at time intervals representing a logarithmic scale. As with future controlled experiments investigating the depth profile of powerboat exhaust HCs, recovery experiments could also benefit from using larger tanks, artificial lakes or mesocosms.

(iii) General Features: Controlled Experiments

Some spillage occurred from the tank during engine operation in all controlled experiments. This varied somewhat, with a mean amount of 15.8 % or 283 litres per experiment (S.D. 3.54 % or 63.25 litres). In general, further reduction of spillage would lead to greater accuracy in future experiments. This could be achieved by either of two means:

1) The engine could be run in a neutral gear. This would have one possible drawback, as the chemical characteristics of engine exhaust could vary with different gear settings.

2) A better solution would involve the fitting of a special propeller which causes no net

movement of water. These propellers are still subjected to the same forces which act on a conventional propeller in forward gear but would cause very little spillage. An engine fitted with this type of propeller may be capable of producing exhaust with the same chemical characteristics as an engine with a conventional propeller.

Before commencing experiments in the 1787 litre tank, it was decided on the basis of previous research (Jüttner *et al.*, 1994, 1995*a*; Montz *et al.*, 1982), that 10 min of engine operation was sufficient to produce water that was sufficiently contaminated for all experiments described in Chapter 5 of the current study. This was proven to be justified.

(iv) Potential Recovery from Powerboat HC Contamination in Field Situations

Because of the effects of water currents and wind, recovery of water quality in the field could be expected to be faster than that which took place in the controlled experiments. However, the actual rate was difficult to estimate from field experimental data from the current study, due to the unpredictable nature of powerboat exhaust HC input in the field. The Boating Industry Associations study (BIA, 1975) estimated that field recovery would be approximately ten times as rapid as that found in controlled experiments, but based this estimate purely on a laboratory experiment in which beakers of exhaust-polluted water were kept in the laboratory under aerated and non-aerated experimental conditions. Half-lives of 12 hours and 5 days were calculated for the HC concentrations in the aerated and non-aerated samples of polluted water, respectively, and it was concluded that recovery in the field would closely parallel that of the aerated test vessel. Details are lacking, but even if their estimate is correct, half-lives of 12 hours or so might be expected in Loch Lomond. This seems to be a very short time period, but as there is likely to be almost constant input of volatile aromatic HCs in some areas of the loch in summer, full recovery of water quality in these areas may not occur until well into autumn, when powered boat numbers decline (Adams *et al.*, 1992).

5.5.5: Introduction of Hydrocarbons to the Water

Controlled experiments in the present study, showed that input of volatile aromatic HCs to the water from engines follows a predictable pattern. Up to aqueous concentrations of about 8 $mg.l^{-1}$, there was an almost linear relationship between the time for which the engine was operated and the volatile aromatic HC concentrations in the water of the 1787 litre stainless steel tank. 8 $mg.l^{-1}$ is a high concentration, very unlikely to occur in Loch Lomond (the highest recorded concentration in the loch in the summer of 1996 was 37 $\mu g.l^{-1}$). Since HC concentrations in Loch Lomond would always be expected to be far below saturation levels, the amount of engine operation in the field should be directly proportional to the amount of HCs introduced to the water. This provides further evidence for the validity of the strong statistical relationship found between local boat densities and surface water volatile aromatic HC concentrations (see Section 5.4.3).

When the engine was run for time periods longer than 10 minutes, saturation of the water with volatile aromatic HCs apparently occurs. Evidence for this is seen in the results of the experiment in which the engine was run for 3 hr. 30 min. (Fig. 5.12). As can be seen, the rate of input progressively slows after 10 minutes and appears to become almost negligible after about two hours. Previous research (Shiu *et al.*, 1988) has shown that the maximum solubility of petrol in water (expressed as the total concentration of soluble HCs from petrol), is 98 $mg.l^{-1}$ at 22°C.

As already mentioned, heterogeneity of HC concentrations in artificially polluted water within the tank may have occurred. This could have been a problem in the investigations of recovery from outboard motor pollution. By contrast, in the experiments investigating the introduction of HCs to the water, the water in the tank was being constantly mixed by propeller action at all times of sampling during engine operation. It is thus conceivable that this mixing allowed greater homogeneity of the water in the samples during these experiments.

Table 5.7: The proportion of fuel consumed that is exhausted from powerboat engines, measured (or estimated) in the present study and previous studies. Some studies have calculated the total proportion of fuel (including gaseous HC emissions) used that is emitted from engines, and this is indicated in Column 4. Other studies have measured the proportion of fuel used that enters the water as dissolved HCs, following engine operation, and this is indicated in Column 5.

Study	*Time of engine run*	*Speed (rpm)*	*% of fuel consumed exhausted from the engine*	*% of fuel consumed entering water*
Present study	10 min	2420-2850 (estimated)	-	4.96
"	"	"	-	4.72
"	"	"	-	3.60
"	"	1800 – 2000 (measured)	-	7.34
Coates and Lassanske (1990)	Continuous measurement.	Wide range of settings, weighted average presented here.	8.30	-
US EPA (1991)	Estimated.	-	25.00	9.38
BIA (1975)	Continuous, measured hourly	3000		2.50

In the present study, it was found that 3.60 % to 7.34 % of the fuel consumed by outboard motors persists in the water, with most of this being volatile aromatic HCs. This finding agrees reasonably well with those of most previous studies (Table 5.7). The Boating Industry Associations (1975) estimate may be conservative, especially as some of the engines tested in that study were considerably more polluting than modern engines. These older engines did not possess crank-case oil recycling devices, which were introduced to most engines from the late 1960s onwards (Muratori, 1968). It is possible that differing ideas of safe environmental concentrations of pollutants coupled with the less sophisticated analytical equipment available at the time in the BIA (1975) study could have contributed to the different conclusions of that study.

Coates and Lassanske (1990) used a sophisticated analytical set-up to quantify total gaseous HC emissions from a modern outboard engine, while the US EPA (1991) only estimated total HC emissions from inventories of engine use. However, the latter study included many old and worn engines in its estimates. These engines have higher emission rates than newer ones, and there is nothing to suggest that the US EPA's estimate is exaggerated.

Further research is necessary to improve the accuracy of measurements of HC output from boat engines, in general, but also in particular, with respect to different fuel and engine types. The fluorescence-based techniques described in Chapter 4 of the current study may not need much modification to be used in the detection, identification and quantification of a wide variety of classes of HCs. Other valuable future research could include the accurate quantification of total (including gaseous) HC emissions from all types of boat engines (using different fuel types), particularly older ones, using similar techniques to those outlined in Coates and Lassanske (1990).

Chapter 6

The Toxicity of Powerboat Exhaust-Polluted Water

6.1: Introduction

6.1.1: Previous Relevant Work – Toxicity of Hydrocarbons

It is well known that hydrocarbons (HCs) are toxic. Connell and Miller (1984) reviewed the toxicity of crude oil and diesel oil (a refined oil, also known as No. 2 fuel oil) to many marine and some freshwater organisms. Table 6.1 summarises these data, and it can be seen that both substances are toxic at low concentrations and also that diesel is more toxic than crude oil.

Table 6.1: The toxicity of crude oil and diesel oil (No. 2 fuel oil) to selected marine and freshwater organisms: lethal toxicity in 96 hr LC50 tests expressed as aqueous HC concentrations in ppm (data from Connell and Miller, 1984; units not specified further).

		96 hr. LC50	
Species Tested	*Life Cycle Stage*	*Crude Oil*	*Diesel Oil*
Cod (*Gadus morhua*)	Eggs (0.5-10 days old)	2.6 - 55.0	
Sheepshead minnow (*Cyrinodon variegatus*)	*Adults*	>19.8	>6.9
Pink salmon (*Oncorhyncus gorbuscha)*	Adults	2.92	0.81
Dolly Varden (*Salvenius malma*)	Smolts	2.94	2.29
Grass Shrimp (*Palaeomnetes pugio*)	Larvae	-	1.2
	Postlarvae	-	2.4
	Adults	-	3.5
Brown Shrimp (*Penaeus aztecus*)	Postlarvae	-	6.6
	Early juveniles	-	3.7
	Late Juveniles	-	2.9
King Crab (*Paralithodes camtschatica*)	Stage I	2.0	-
	Stage I (moulting)	1.3	-
Polychaete worms (*Neanthes arenaceodentata*)	Juveniles	15.0 -19.9	4.0 - 8.4
	Adults	12.5 -17.6	2.0 - 4.2

There have also been studies in which the aquatic toxicity of single HC compounds found in petroleum was examined. In a large-scale review of marine oil pollution (NRC, 1985), the results of toxicity tests investigating the response of several standard marine test organisms to a wide range of hydrocarbon (HC) compounds found in crude oil were presented (see Table 6.2). Different classes of HC compounds have different toxicity, but it was found that, due to their abundance and relatively high water solubility, the compounds responsible for much of the toxicity of crude oil were aromatic HCs. These include both volatile aromatics with one benzene ring which are moderately toxic, weight for weight but form a high proportion of the total weight of crude oil (John and Soutar, 1981) and Polycyclic Aromatic Hydrocarbons (PAHs), which are highly toxic but only form a small proportion of the

total weight of crude oil (NRC, 1985). Most of the organic compounds entering water from outboard motor exhaust are volatile aromatic HCs (Jüttner *et al.*, 1995*a*).

Table 6.2: The toxicity of particular HC compounds (including volatile aromatic HCs and PAHs) found in crude oil to selected marine and freshwater organisms. Results are expressed as the LC50s in ppm (data from NRC, 1985; units not specified further). Different columns indicate different test organisms. Underlined values indicate a 24 hr exposure period, whereas the remaining values are end points of 96 hr LC50 tests.

Compound	Type of Compound	*Crago*	*Cancer*	*Cyprinodon*	*Palaeomnetes*	*Amphipod*	*Striped Bass*
Benzene	Volatile aromatic	20.0					5.8
Toluene	Volatile aromatic	4.2	28.0				7.3
1,3 DMB	Volatile aromatic		12.0		3.5		9.2
1,4 DMB	Volatile aromatic	2.0					2.0
Trimethyl-benzene	Volatile aromatic		5.0		5.3	4.4	
Naphthalene	PAH			2.0	2.2	2.3	
Methyl-naphthalene	PAH		1.8	3.3	1.1		
Fluorene	PAH			1.6	0.3		
Phenanthrene	PAH				0.4		

Although, a number of previous studies, using fish as test organisms, have shown that outboard motor exhaust-polluted water (EPW) is toxic (Brenniman *et al.*, 1979; Kuzminski *et al.*, 1972; English *et al.*, 1963), as yet, there has been only one published experiment (see Jüttner *et al.*, 1995*b*) carried out using *Daphnia magna*, a standard toxicity test organism (Baird *et al.*, 1989*a*). Jüttner *et al.* (1995*b*), did indeed show that outboard motor-EPW was toxic to *Daphnia*, although details of the measurement of hydrocarbons in the toxicity tests are lacking.

6.1.2: Toxicity Tests

In order to assess the toxicity of any substance (including EPW), an appropriate laboratory toxicity test is necessary. All such toxicity tests involve subjecting test organisms to a range of different dilutions of the substance to be tested. A number of standard methods have been developed (see Rand, 1993) and the resulting toxicity tests enable comparison of the toxicity of different substances.

Toxic effects can be either direct or indirect. The former includes toxic effects induced by the toxicant itself (such as narcosis, mutagenesis and irritancy), while the latter is toxicity due to adverse changes in the environment of the test organisms, one of the most important being changes in oxygen levels (Rand *et al.*, 1993). Most toxicity tests aim to determine the direct toxicity of a substance and to do this, the test can only be valid if no indirect effects occur.

Toxicity tests can be subdivided into two categories: tests that measure acute effects, which are usually evident within a short time period of exposure (less than 4 days), and those which measure chronic effects, evident after a relatively longer period of exposure, usually of at least several weeks (Rand *et al.*, 1993).

All toxicity tests aim to determine an Effect Concentration (EC). This is the concentration of a tested substance having an effect on a specified proportion of the test organisms. EC's include the EC0, EC100, and EC50, which are: the highest concentration effecting no test organisms; the lowest concentration effecting all test organisms; and the concentration effecting 50 % of test organisms, respectively. The most statistically reliable and widely used EC is the EC50 (Wardlaw, 1985), which is the concentration of the chemical tested that results in the response of 50 % of test organisms over a specified exposure time (usually 24 or 48 hours). If, in a particular test, the elicited response of the organism is death, the EC is the Lethal Concentration (LC).

6.1.3: Previous Work: Standardisation of LC50 Toxicity Tests

In recent years, LC50 toxicity tests have undergone increasing standardisation, both with respect to the organisms used and test conditions (Persoone and Janssen, 1993) which allows meaningful comparison of the toxicity of various substances. Among the most widely used test organisms for aquatic pollutants in freshwater are the daphnids (water fleas) - crustaceans of the genus *Daphnia* and related species (Baird *et al.*, 1989*a*; Soares *et al.*, 1992). This group of test organisms has numerous advantages:

(1) Test procedures have been internationally standardised.

(2) The test organisms respond predictably to toxicity.

(3) They are sensitive to a wide variety of pollutants.

(4) They have a widespread distribution in aquatic systems throughout the world.

(5) They occupy a key position in aquatic food chains, intermediate between phytoplankton and fish

(6) They are relatively easily cultured in laboratory situations and their small size and rapid generation time means that little bench space and laboratory time are required.

6.1.4: Chronic Toxicity: No Observable Effect Concentrations (NOECs)

Chronic toxicity is usually estimated from an EC0, measuring a sublethal effect such as feeding rate or reproduction to determine the highest concentration of the chemical that does not effect the measured function over a specified time. A

variety of time intervals can be used, depending on the function measured. This EC0 is then termed the NOEC (No Observable Effect Concentration).

To determine a pollutant's NOEC, measurable sublethal effects need to be investigated in a controlled experiment. Some of the most reliable NOEC tests involve measurements of the reproduction rate of *Daphnia magna*. In a large-scale study, Kuhn *et al.* (1989*b*) calculated NOECs by means of a 21 day reproduction test for wide variety of toxicants. Although quicker tests, lasting less than 7 days, have more recently been developed (Baird *et al.*, 1991), a faster and simpler alternative was sought for the present study, due to time constraints in setting up and carrying out such experiments. Such a method of determining a NOEC involves conducting a controlled feeding trial with *Daphnia magna*, for a set period of time, typically 24 or 48 hours. Daphnids are particularly appropriate test organisms as they feed unselectively (Waddell, 1993) and have a predictable relationship between feeding rate and concentration of food particles in the surrounding medium (Bohrer and Lampert, 1988). However, when an appropriate stress is introduced (such as a toxicant), feeding rate declines. This has been shown to be a sensitive and predictable measure of toxic effects (Jones *et al.*, 1991; Ferrando and Andreu, 1993).

6.1.5: The Context for This Study

So far in the current study, the chemical characteristics of outboard motor exhaust-polluted water (EPW), produced by an appropriate method under controlled conditions (see Section 3.3, in Ch. 3) have been ascertained. Measurable concentrations of these compounds have also been found at many sites in Loch Lomond in summer during periods of high powerboating activity (Ch. 5, Section 5.4.3). The next obvious step in the current study was to see if the levels of these compounds in Loch Lomond could pose a risk to the aquatic environment. To do this, I examine the toxicity of outboard motor EPW (Exhaust-Polluted Water), explore the concept of a theoretical safe level of powerboat exhaust HCs and compare this with the concentrations of these contaminants in Loch Lomond in summer.

6.1.6: Aims

1) To define an LC50 of aqueous outboard motor exhaust pollutants for the standard test organism, *Daphnia magna.*

2) To ascertain the toxicity of a water-accommodated fraction (WAF) of outboard motor fuel (petrol:oil mixture), and compare the aquatic toxicity of fuel before and after combustion.

3) To investigate the volatility of outboard motor exhaust pollutant HCs and the effect of this on toxicity tests.

4) To compare the sensitivity of a test species from Loch Lomond (*Diaptomus gracilis*) to outboard motor EPW with that of *Daphnia magna.*

5) To investigate the recovery rate of water quality following outboard motor pollution, using *D. gracilis.*

6) To estimate a theoretical safe level of powerboat exhaust pollutants for Loch Lomond, using the results of the LC50 and NOEC toxicity tests performed in the present study.

6.2: Methods

6.2.1: Glassware Washing

All glassware used in both in the rearing of *Daphnia magna* and toxicity tests was thoroughly cleaned before any tests or culturing were carried out as described in Section 3.2.1.1. This served not only to clean the glass, but also to remove any traces of hydrocarbon contaminants. Due to time constraints, the full procedure was not feasible each time glass needed cleaning and was done fully only every fourth occasion. On other occasions, an abbreviated sterilisation procedure was carried out, whereby the glass was just bathed in "Decon 90", then rinsed four times in tap water and distilled water and subsequently air dried.

6.2.2: Culturing *Daphnia magna*

*6.2.2.1: Culturing of Algal Feed (*Chlorella vulgaris*) for* D. magna

In the present study, it was necessary to set up a culture of *Chlorella vulgaris* (strain K12) to produce enough algal cells for an appropriate feeding regime for a *Daphnia magna* culture. Before setting up the *C. vulgaris* culture, all glassware was cleaned, using the procedure previously outlined in Section 6.2.1. Rubber tubing was further cleaned by washing in reagent grade methanol and air drying, using an air pump.

Like all plants, algae have three main requirements to photosynthesise, grow and multiply: adequate nutrients, a carbon source and adequate light (Keeton and Gould, 1986). To provide the required nutrients at the correct concentrations, an appropriate inorganic medium – modified Katz and Meyers medium (see Dominy and Williams (1986) - was made up, containing all essential nutrients and minerals. Table 6.3 shows all of the constituents, their stock solutions and the amounts of each necessary to produce the working medium. In the current study, glass-distilled water

was used to make up both the stock solutions and the working medium.

Table 6.3: Composition of the working *Chlorella vulgaris* culture medium (modified Katz and Meyers medium) and its stock solutions used in the present study.

Compound	Concentration in stock solution ($g.l^{-1}$)	Amount of each stock solution needed to make 250 ml.	Concentration in medium ($g.l.^{-1}$)
KNO_3	25.0	10 ml	1.00
K_2HPO_4	5.0	10 ml	0.20
KH_2PO_4	5.0	10 ml	0.20
$MgSO_4.7H_2O$	5.0	10 ml	0.20
$CaCl_2.2H_2O$	2.5	10 ml	0.10
EDTA-disodium salt	0.5	10 ml	0.02
Metals 45*	Neat powder	5 mg	0.02

*Added as a mixed powder. Constituents are as follows, in percentages by weight:

Ferrous Ammonium Sulphate	63.8 %
$ZnSO_4.7H_2O$	20.0 %
$MnSO_4.H_2O$	7.1 %
$CuSO_4.5H_20$	1.4 %
$CoSO_4.7H_20$	2.2 %
H_3BO_3	2.6 %
$(NH_4)6Mo_7O_{24}.4H_20$	2.9 %

To set up the initial algal culture, 500 ml of freshly-prepared culture medium was poured into a 1 litre narrow neck clean glass bottle and inoculated with a small amount (approximately 1 mg) of a pure plate culture (including some viable cells) of *Chlorella vulgaris* K12. Subsequent cultures were sub-cultured from this first one, using approximately 0.5 ml of the liquid medium, after maximum cell density was reached, which took approximately two weeks (± two days).

An appropriate Carbon source was introduced by pumping air, filtered by a 0.22 μm "Millex" 6V Millipore sterile filter unit at a rate of 1000 $ml.min^{-1}$ (equivalent to 2000 $ml.min^{-1}.l^{-1}$) into the culture. The filtration process was necessary to prevent contamination of the medium from dust and bacteria. As a further safeguard against such contamination and to minimise evaporation, the mouth of the 1 l culture bottle

was securely sealed with sterile cotton wool and then wrapped up with tinfoil. Evaporation did occur to some extent but the volume of the culture medium was maintained by twice weekly topping up with glass-distilled water, delivered through a sterile glass Pasteur pipette, fitted through the tinfoil and cotton wool barrier at the mouth of the culture vessel. This pipette also had a removable tinfoil "lid". Fig. 6.1 depicts the algal culture set-up used in the current study.

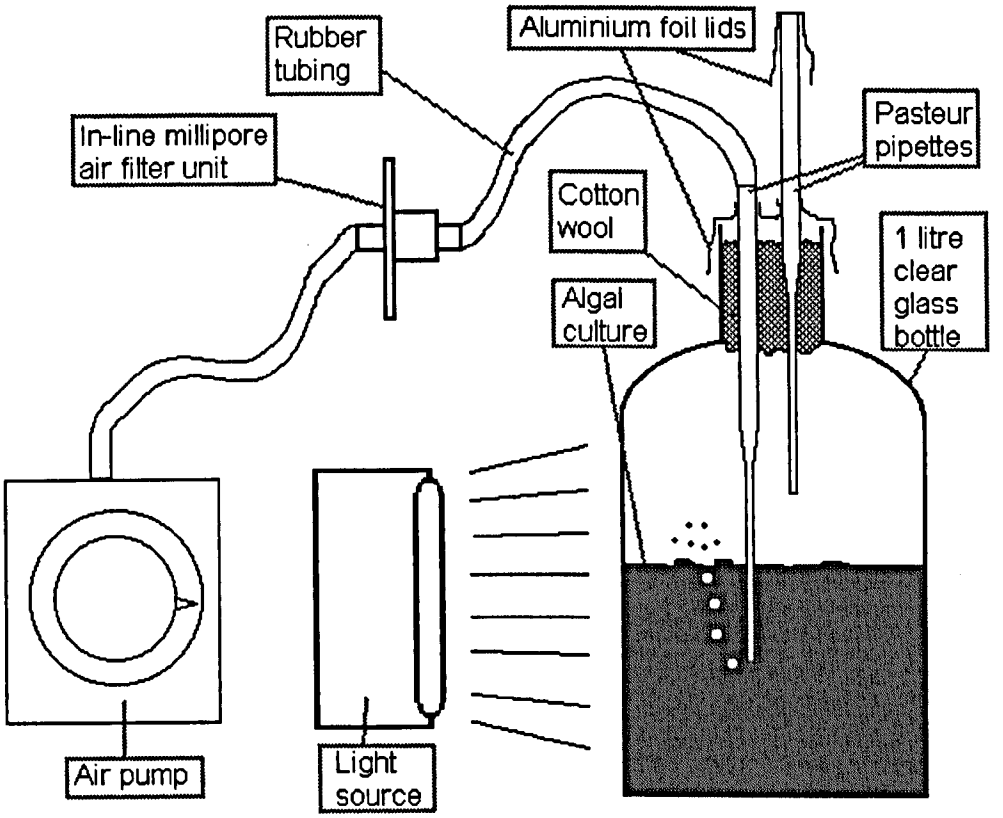

Fig. 6.1: Apparatus used to culture Chlorella in this study.

The culture was maintained at 20 to 25°C at constant illumination by a 12 W fluorescent light bulb, which itself gave an irradiance of approximately 1/30 of full sunlight (15 Wm^{-2}). Although excessive light intensity can reduce growth rate, a phenomenon known as photo-inhibition (Barber and Andersson, 1992), it was found that the optimum growth of the algal cultures was achieved by siting them in the brightest part of the laboratory, approximately 1 m from a South facing window. This gave a minimum light intensity of 15 $W.m^{-2}$ at night (with all light emanating from the artificial light source) and a maximum of approximately 265 $W.m^{-2}$ (which is

approximately 250 $W.m^{-2}$, or half full sunlight in summer in Scotland, plus the output of artificial light), during summer daylight hours (all of these cultures were made up during June and July 1997).

The final cell density of the cultures ranged from 6.8×10^7 to 1.66×10^8 $cells.ml.^{-1}$. This was counted using a "Nebauer" haemacytometer, with a $0.1mm^3$ counting chamber, measuring 1 mm x 1 mm x 0.1 mm in depth. The stock algal cultures were then stored in closed plastic vessels, in the same incubator as the *Daphnia* cultures where they were viable (and still usable as feed) for up to 2 weeks after termination of culturing (Dominy and Williams, 1985).

6.2.2.2: Procedure of Daphnia magna *Culture*

In order to have an adequate supply of toxicity test organisms for the present study, a culture of *Daphnia magna* was set up in June 1997 and maintained for the duration of testing (8 weeks). Firstly, sixty female *Daphnia magna* (Clone Beak) neonates were transported from the Institute of Aquaculture, Stirling University to the University Field Station, Rowardennan. Transfer time was less than two hours with the animals otherwise kept in constant conditions: a temperature of 20±1°C and a light regime of 16 hours light and 8 hours of darkness (16L:8D). The medium was ASTM (American Society for Testing and Materials) hard water (ASTM, 1980*b*), which is an appropriate medium for the rearing of *Daphnia magna* and the conduction of toxicity tests. Table 6.4 shows the composition of the working medium and the stock solutions and the amount of each required to make 10 litres, by diluting in suitably pure water. Glass-distilled water was used in the current study.

Recent research (Baird *et al.*, 1989*b*) has shown that growth and reproduction is improved by adding an appropriate quantity of *Ascophyllum* (a marine seaweed) extract. In the present study, this was done by adding 2 ml of a working solution of seaweed extract to each litre of culture medium. In turn, the working solution was made up by diluting the concentrated seaweed extract by 140, using distilled water.

More details of the benefits of such organic additives to cultures of *Daphnia magna* are given in Baird *et al.* (1989*b*).

Table 6.4: Composition of ASTM hard water, its stock solutions and the quantity of each needed to make 10 litres of working medium.

Compound	Concentration in stock solution	Amount of each stock solution needed to make 10 l of working medium	Concentration in working medium
$CaSO_4.2H_2O$	2.4 $g.l^{-1}$	500 ml.	120.0 $mg.l^{-1}$
KCl	1.6 $g.l^{-1}$	50 ml.	8.0 $mg.l^{-1}$
$NaHCO_3$	38.4 $g.l^{-1}$	50 ml.	192.0 $mg.l^{-1}$
$MgSO_4.7H_2O$	49.1 $g.l^{-1}$	50 ml.	245.5 $mg.l^{-1}$
Total hardness			140.0 $mg.l^{-1}$, expressed as $CaCO_3$

The medium was changed twice a week (which approximates to each occasion of neonate release), following standard procedures (Baird *et al.*, 1991; Taylor *et al.*, 1998). Other than the transfer period, animals were kept in 2 litre glass beakers, 20 to each beaker. The beakers were covered in tinfoil to prevent entry of dust and contaminating bacteria and kept in a "Gallenkamp IH 277" cooled incubator at 20±1°C, with a light regime of 16L:8D, controlled by an electric timer. The light level was maintained at 500 lux (a standard level – see Cooney, 1993) by means of two 8 W fluorescent bulbs. The daphnids were fed at each medium change, with a pure culture of *Chlorella vulgaris*, which is a spherical, solitary, unicellular green alga, with a mean diameter of 3-5 μm. The density of algal cells was kept at an optimum concentration for rearing, 5 x 10^5 cells ml^{-1}, by the addition of 5 to 15 ml of a concentrated stock solution (itself ranging from 6.8 x 10^7 to 1.66 x 10^8 cells ml^{-1}) to each beaker at the times of medium change.

These rearing conditions satisfy the guidelines outlined in Goulden *et al.* (1982) and were necessary to maintain a healthy reproductive culture. Any serious alteration of these conditions may cause sexual reproduction, which destroys the purity of the clone in question, invalidating the results. The neonates (asexually

reproduced young, genetically identical to the adults) are released in clutches of 10-20 animals per adult female over 12 hours, about every 3 days with neonates less than 24 hours old used as test organisms. In practice there was variation of the timing of release among the 60 breeding animals kept and neonates tended to be released most days in the culture, with peak release occurring every 3 days. Consequently, the neonates were removed every day in order to ensure that those collected for testing were always 0-24 hours old. In the case of all adults, only neonates from the second brood onwards were used in toxicity tests. This is a standard procedure (see Goulden *et al.*, 1982).

6.2.3: LC50 Estimation Methods – *Daphnia magna*

6.2.3.1: Test Substances

The substances used in LC50 tests in the current study included outboard motor EPW and outboard motor fuel:oil mixture Water Accommodated Fraction (WAF). The EPW was produced as described in Section 5.3.2.4(b), while the WAF was produced by vigorous agitation of 50:1 petrol:oil mixture in ASTM hard water (more details of this are found in Section 6.2.7). Both substances were used unfiltered in toxicity testing, as this was felt to be the most ecologically relevant way of exposing test organisms to such a complex mixture of hydrocarbons. Samples of complex effluents are usually tested unfiltered (Dorn and van Campernolle, 1993) and in this respect, both substances were treated as effluents.

6.2.3.2: Acclimatisation of Daphnia magna *to Loch Lomond Water*

Clone Beak *Daphnia magna* were used as test organisms in the study described here. They have a water hardness requirement of 140 $mg.l.^{-1}$ (expressed as the equivalent concentration of $CaCO_3$), but the hardness of water from Loch Lomond is much lower, normally being in the range 10-20 $mg.l.^{-1}$ (Best and Traill, 1994) and a

sudden change of this order would place a lot of stress on *Daphnia* neonates unaccustomed to such soft water (Cooney, 1993). In the current study, it was not feasible to obtain a strain of *D. magna,* which was innately suited to such soft water conditions and it would have been far too laborious to attempt to correct the hardness of the EPW (produced by running an outboard motor in a tank filled with Loch Lomond water) to the same level as that preferred by Clone Beak *D. magna.* It was felt that the best way to overcome this problem was by acclimatising adult *Daphnia* to loch water before taking neonates subsequently released. Neonates thus produced are suitable for toxicity testing at low hardness levels. This acclimatisation process is a standard procedure in the toxicity testing of effluents, which are often complex mixtures (Dorn and von Campernolle, 1993).

To produce neonates accustomed to low hardness, breeding adults kept in ASTM hard water were gradually transferred to filtered (by Whatman No.2 filter paper) water from the Field Station's Loch Lomond water supply. This water was taken from a depth of 2 m in the Field Station Bay in Loch Lomond (Ordnance Survey Grid Reference NS 377 957). The filtration process removed almost all organisms from the loch water, and largely prevented any changes in water chemistry in the short-term (up to 1 week) and is a standard procedure (Habib, 1993). Fresh supplies of filtered loch water were obtained weekly. The transfer of adult *Daphnia* took place in three steps, over 48 hours:

1) Firstly from pure ASTM hard water to a solution of 33 % filtered loch water and 67 % ASTM hard water.
2) 24 hours later, to 67 % filtered loch water and 33 % ASTM hard water.
3) Finally, after another 24 hours, to 100 % filtered loch water.

The WAF was made up by dissolving the outboard motor fuel:oil mixture in ASTM hard water, and acclimatisation of Daphnids to Loch Lomond water was consequently unnecessary.

6.2.4: Changes in HC Concentration During Toxicity Tests

6.2.4.1: Measurement of HC losses: high initial concentrations

In order to measure the losses of volatile aromatic HCs from the test media during toxicity testing, samples of the media were taken before, during and after toxicity tests in Experiments 2 and 3 (see Sections 6.2.6 and 6.2.7 respectively). Before each of these five tests, there was more than an adequate volume of the neat artificially contaminated medium available for the replicates in each test to be produced and to allow a 100 ml sample to be taken for HC analysis. This corresponded to the "zero time" sample in each LC50 test. Preliminary tests suggested that all test organisms would be very likely to be dead after 24 hours and one of the actual test vessels was used for HC analysis at this stage in testing. After 48 hours, another of the 100 ml vessels was used to investigate changes in HC concentration over that time period.

Sample processing was carried out as described in Ch. 3, Section 3.2.1.3, except that the final sample volume was 5 ml (not 1 ml). Sample purification was performed using the conditions outlined in Table 4.3 and HC analysis was by fluorescence spectroscopy (see Table 4.1). For the samples of media taken after 24 and 48 hours, the entire contents of the test vessel (including test organisms) was used for this analysis.

HC losses were high from all EPW and WAF tests. Fig. 6.2 demonstrates the decline in HC concentration of the undiluted test substances (EPW or WAF), with all values expressed as percentages of the initial HC concentration in each test. This decline was found to be highly statistically significant ($H = 12.10$; 2 d.f. ; $p = 0.002$; Kruskal-Wallis one-way ANOVA).

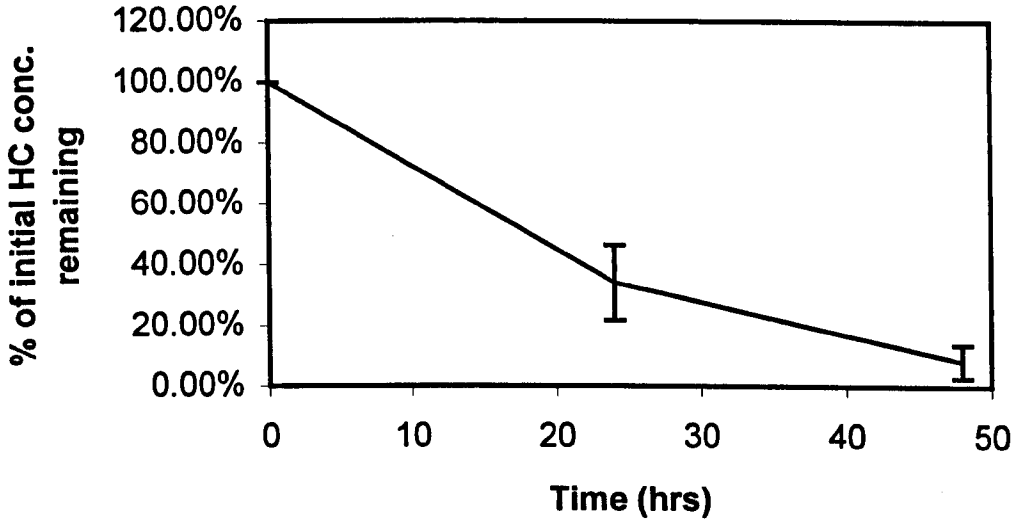

Fig. 6.2: Decline in HC concentration in the media of five LC50 tests (two WAF and three EPW), expressed as percentages of the initial concentration. Mean decline is shown, with S.D. bars.

6.2.4.2: Measurement of HC losses at a range of initial concentrations

In two of the tests in Experiment 2, an additional analysis was carried out in order to examine the losses of HCs from the test media at lower initial concentrations. To do this, all of the replicates including the controls (see section 6.2.6) were subjected to HC analysis before and after (48 hr.) testing.

HC losses were high, with a very similar proportional loss at all initial concentrations of EPW. Fig. 6.3 demonstrates this, by graphically comparing initial and final concentrations of volatile aromatic HCs in the test media. HC concentrations in the control vessels were very low and remained unchanged in one test, but rose slightly in the other.

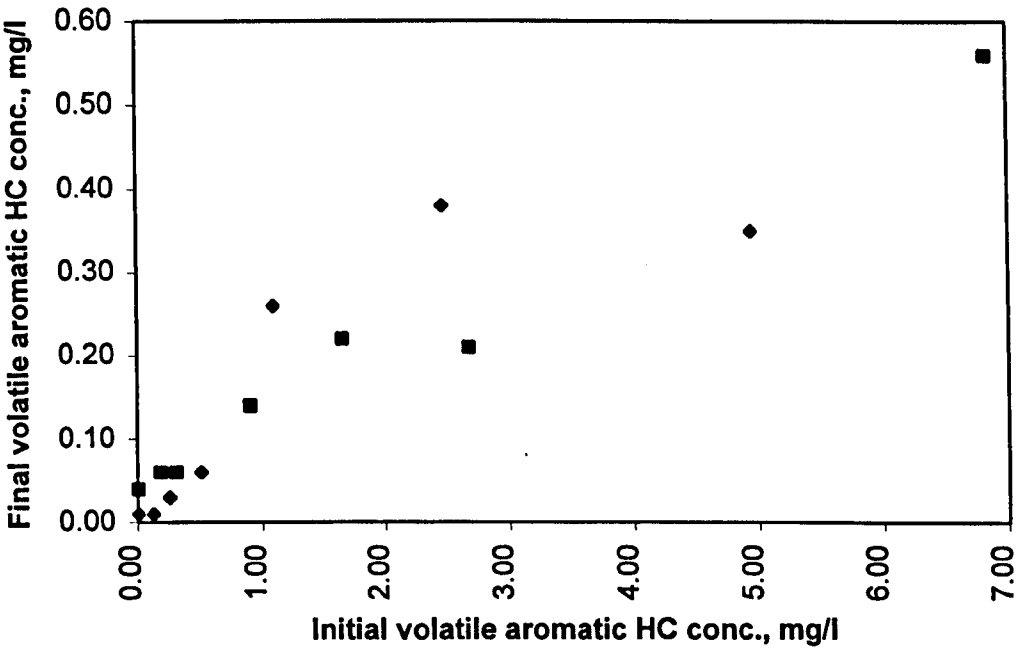

Fig. 6.3: Comparison of initial and final HC concentrations in EPW LC50 test media in Experiment 2, at a range of initial concentrations. The square symbols represent one test, while the diamond symbols represent the other.

6.2.4.3: Validation of Dilution Procedure in EPW LC50 Tests

These measurements of volatile aromatic HCs from the test media were also used to investigate the accuracy of dilution of EPW before testing. The actual concentrations, measured by fluorescence spectroscopy (see Tables 4.1 and 4.3) were compared to the intended concentrations.

The dilution procedure was reasonably accurate. Although this was done in only two of the fourteen replicate tests in which HCs were measured, the results were consistent enough to suggest that the procedure was carried out correctly in all tests. Before statistical analysis, it was necessary to:

I. arcsine transform the data in each experiment, as it is in the form of proportions.
II. investigate whether the data from the two experiments could justifiably be combined for analysis.

Using Analysis of Covariance, it was found that statistically, the transformed data could indeed be pooled [as there was no significant difference between the slopes ($F_{1,6}$ = 3.3, n.s.) or elevations ($F_{1,7}$ = 0.28, n.s.) of the two regression lines].

Combining the transformed data, it was found that there was a high correlation between the expected concentrations (found by multiplying the actual measured concentration of the undiluted tested substance by the intended dilution factor) and the actual concentrations (t = 16.18, $p<0.001$, 9 d.f. r^2 = 96.7 %; regression analysis). There was some slight variation indicating that exact serial dilutions were not quite produced.

6.2.4.4: Oxygen Concentrations in Toxicity Test Medium

As it was intended to measure the direct toxicity of EPW, dissolved oxygen, being the best indicator of any indirect effects (Cooney, 1993) was measured in one of the EPW tests. A "Strathkelvin" No. 1302 oxygen electrode, coupled with a "Strathkelvin" Model 781 oxygen meter, was used to measure dissolved oxygen in each of the three replicate vessels, at three concentrations (100 %, 12.5 % and the controls). It had to be assumed that these 3 replicate O_2 measurements were representative of the full range of concentrations of EPW.

At no time did measurements fall below 86 % of the saturation concentration at the requisite temperature. These measurements easily fulfill the criteria detailed in OECD (1984), in which aqueous oxygen concentrations in test media are required to be at least 60 % and strongly suggest that oxygen levels were not causing any indirect toxicity.

6.2.4.5: Additional Toxicity Test Conditions Specific to the Current Study

In pilot studies, it was found that *Daphnia* floated to the surface of the test medium when exposed to EPW. Such "floaters" complicate the interpretation of toxicity test data, as their exposure conditions are difficult to determine (Dean and DeGraeve, 1986). It was decided that the best solution to this problem was the production of screen devices to prevent the animals floating to the surface and produce a more realistic exposure to EPW (see Fig. 6.4). These screens were produced by fixing cotton meshes of 0.5 mm diameter around the mouth of each beaker, using rubber bands. These closed beakers were in turn placed in larger vessels (2 litre beakers, or Kilner jars) which were filled with the same diluted EPW as the smaller vessels. The controls, with no HC pollutants but possessing the screen devices, were also set up in this way. Similar devices have been used in previous studies (Dean and DeGraeve, 1986).

Before all testing, the cotton mesh screens were washed in a washing machine before being rinsed thoroughly in distilled water before testing, to remove any soap residue. In addition, only clean rubber bands (not previously used) were used to produce these devices.

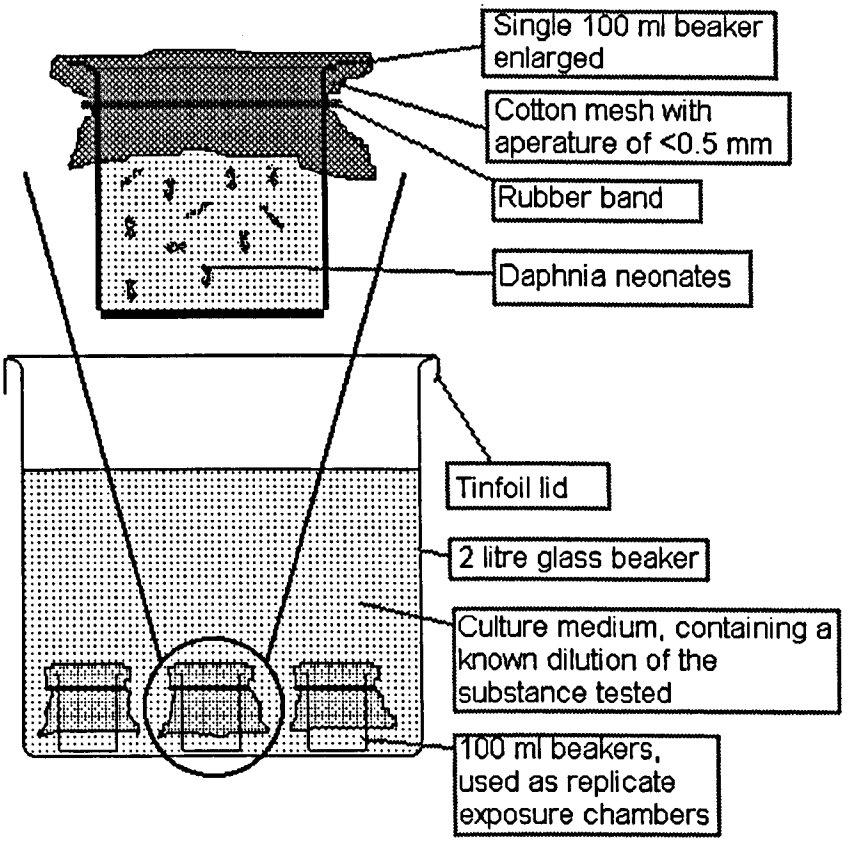

Fig. 6.4: *Screen devices to prevent* Daphnia magna *from floating to the medium surface during toxicity tests.*

6.2.5: Experiment 1 - Preliminary Toxicity Experiments on EPW, using *Daphnia magna*

In this test, exhaust-polluted water (EPW) was collected from the 510 litre concrete experimental tank following outboard motor operation, with surface water samples taken using the procedure described in Ch. 3, Section 3.2.1.2. Six serial dilutions, ranging from 0.1 to 100 % of the strength of the polluted water from the tank were made, using a dilution factor of $\sqrt{10}$. This is a standard dilution factor, commonly used in previous studies (e.g. Kuhn *et al.*, 1989*a*). A control was also set up, using pure filtered Loch Lomond water. Three replicates were set up for each concentration of EPW (and also the control), with ten neonates (previously acclimatised to Loch Lomond water), each less than 24 hours old, placed in each 100 ml beaker. Daphnids were acclimatised to Loch Lomond water before testing, according to the procedure in Section 6.2.3.2. The test organisms were then housed in

a Gallenkamp cooled incubator, under the same controlled temperature and light regimes as those used for rearing neonates (see Section 6.2.2.2). No feeding or renewal of the test medium took place during the test and the mortality rate was determined after 24 and 48 hours. Immobile organisms were counted as dead, as immobility gives a good indication of mortality in Daphnids (see Khangarot and Ray, 1989).

All test procedures conformed as closely as possible to those outlined in OECD (1984) and Persoone and Janssen (1993).

The LC50 was calculated on the basis of the HC concentrations in the medium in the test vessels before the test was commenced. Since HC analysis techniques were not available for this particular experiment, the concentrations needed to be estimated as the ratio of the amount of fuel used to the amount of dilution water in the experimental tank (in which the engine was run). Before accurate analytical techniques for HC analysis in water were developed, this was a widely used technique (see Brenniman *et al.*, 1979; English *et al.*, 1963; Kuzminski *et al.*, 1972).

6.2.6: Experiment 2 - LC50 Toxicity Experiments using *D. magna*, with HC Concentration

In this experiment an attempt to find the LC50 of outboard motor EPW was made, in conjunction with direct measures of HC concentrations in the test exposures. Three such LC50 tests were carried out, with EPW produced as described in section 5.3.2.4(b). Despite the larger volume of water in the new tank (1787 l, compared to 510 l), the time of engine operation was left unchanged from the earlier experiments, at 1 hour.

Daphnids were acclimatised to Loch Lomond water before testing, as described in Section 6.2.3.2, while the range of dilutions of EPW was made up using a dilution factor of 2 (which, along with $\sqrt{10}$ is also a standard dilution factor – see Kuhn *et al.*, 1989*a*). Aqueous volatile aromatic HCs were measured in the test media before, during and after toxicity testing as described in Section 6.2.4, using the analytical procedures described in Chapter 4. All samples of medium were 100 ml in volume.

It was intended to produce three replicate sets of test vessels for each concentration of EPW, in each of the three tests here described. However, in the first test, only two replicates were possible, as the supply of neonates for testing was limited at that time. This still fulfils the minimum standard requirements for daphnid toxicity testing (Persoone and Janssen, 1993).

6.2.7: Experiment 3 – WAF (Water Accommodated Fraction) *Daphnia magna* LC50 tests

The toxicity of a Water-Accommodated Fraction (WAF) of the 50:1 fuel:oil mixture was also measured. A WAF includes dissolved HCs and some stable emulsions of HCs in water (Betton, 1993). Previous studies on marine oil pollution have often involved the production of crude oil WAFs, using a wide variety of methods, including slow agitation (NRC, 1985) and drip-through systems (see Carls and Rice, 1988). It was felt that such methods would have been inappropriate in the present study, as petrol is lighter, much less viscous and a lot more volatile than crude oil (Shiu *et al.*, 1988). The procedure used in the present study to create a fuel:oil mixture WAF was based on standard methods for the extraction of hydrocarbons from water using organic solvents (Law *et al.*, 1988; MEWAM, 1988), but applied in reverse, being in effect, the extraction of HCs from the fuel:oil mixture into water.

In the present study, the WAFs were produced by vigorously shaking 2 ml of petrol:oil mixture and 1 litre of ASTM hard water in a large separating funnel for 1 minute. 2 ml (or 1.64 g) per litre was considered to be adequate as the maximum solubility of petrol in water is less than one tenth of this (Peterson, 1994). The solution was allowed to settle for 1 hour to let any excess petrol (which was always present) to float to the surface. The WAF was then poured off by opening the separating funnel tap.

In all two complete LC50 tests were carried out using the fuel:oil mixture WAF as a test substance. Since the WAF was made up using ASTM hard water, there was no need to acclimatise the Daphnids to Loch Lomond water. In both tests, serial dilutions were produced with a dilution factor of 2 as described in Section 6.2.3.2. In addition, there were ten organisms per vessel, three replicate sets of vessels in each test and screen devices used.

The test medium was analysed for volatile aromatic HCs before, during and after LC50 testing, as described in Section 6.2.4.1.

6.2.8: Experiment 4 - LC50s using *Diaptomus gracilis*

Diaptomus gracilis is the dominant zooplankton species in Loch Lomond and is found in large numbers in surface water at all times of the year. They are also easy to obtain, the adults being easily collected, identified and sorted. To obtain animals for testing, zooplankton samples were collected by towing a plankton net for approximately 400m behind a row-boat in the Field Station Bay, in the mid-basin of Loch Lomond (Ordnance Survey Grid Reference: NS 377 955). *Diaptomus gracilis* were subsequently sorted from other plankters under a dissecting microscope at 10 times magnification. To avoid variation in the test results due to sex and ontogenetic difference, only mature females (which were much more abundant than the males and thus easier to collect) were used. Unlike the laboratory reared *Daphnia magna* culture, genetic differences occur in these wild organisms and it was thus desirable to set up a

greater number of replicates of test organisms in each test. Thus five replicates at each concentration were prepared, thereby increasing the potential precision of the results.

In order to minimise stress to test organisms, the test medium used was filtered (Whatman No. 2) surface water from Loch Lomond taken from the same location as the test organisms and the temperature and photoperiod chosen for each test simulated the ambient temperature and photoperiod (see Table 6.5). The EPW concentrations prepared in all tests using *D. gracilis* as test organisms were: 100 %, 50 %, 20 %, 15 %, 10 %, 5 %, 2 % and a control (0% EPW).

In all of the *D. gracilis* tests (as in the preliminary *D. magna* tests), EPW was produced in 510 litre concrete tank, as described in Chapter 3, section 3.2.2.

Table 6.5: Physical/chemical parameters of media used in *Diaptomus gracilis* LC50 tests. All of the conditions were ambient at the time of sampling. Column 1 shows the time for which Exhaust-Polluted Water (EPW) was allowed to stand, after running the engine and before being tested for toxicity.

Time after running engine (days)	*Date of commencement of test*	*Light Regime*	*T°C*	*pH*	*Dissolved O_2 % saturation*
0	13.4.94	14L:10D	6.0	6.15	91
5	18.4.94	14L:10D	7.0	6.31	100
43	25.5.94	17L:7D	11.0	5.98	91

6.2.9: Experiment 5 - Recovery of Water Quality Following Outboard Motor Pollution

In further experiments to investigate recovery of EPW, two further LC50s were performed on *D. gracilis*, using exactly the same procedures described above (Section 6.2.8), with the same EPW concentrations, the same number of organisms per vessel and the same number of replicates in each test. In these two tests, EPW produced, five days and 43 days previously was used (see Column 1, Table 6.5).

6.2.10: Statistical Analysis of Results

All LC50s and their 95 % Confidence Intervals (C.I.) were calculated using the Probit method (see Finney, 1971 and Wardlaw, 1985, for a fuller description). Probit analysis is carried out by plotting the $\log_{10}$ of the concentration of the toxicant against the probit of percentage mortality (estimated from statistical tables). These probit values are then used to give a straight line plot of the log of the concentration versus the probit of mortality. The log of the LC50 is the x-axis value corresponding to the probit value of 5 and the LC50 is found using the antilog of this value. The 95 % Confidence Intervals (C.I.) are calculated by the equation:

$$95\ \%\ \text{C.I.}\ (\log_{10}\text{LC50}) = 1.96(\text{se}(\log_{10}\text{LC50})) = 1.96(1/b\sqrt{pnw_{(av.)}})$$

where

se = the standard error of the estimate for the LC50

b = the slope of the log-dose/probit response line

p = the number of dilutions in the titration

n = the number of animals in each group

$w_{(av.)}$ = the average *weight* of the observations (the relative importance of them). These values are gained from statistical tables.

Two measures of 95 % C.I. were gained. Firstly, the 95 % C.I. of the LC50 was calculated using the above method, with the data pooled from all the replicates in each test. Another source of variation occurs between replicates, and this was measured by calculating LC50s for each replicate independently and calculating the 95 % C.I. between replicates (in this case, using the standard procedure of multiplying the standard deviation by 1.96 – see Fowler and Cohen, 1990).

An appropriate DOS-operated computer programme was used to calculate LC50 values for each test, using probit analysis. Use of such computer programmes for probit analysis is widely reported in previous studies (Wardlaw, 1985; Cooney, 1993; Larsson *et al.*, 1981; Taylor *et al.*, 1998).

In tests where no partial kills occurred (all the test concentrations manifested either total mortality or total survival), there is a standard method for estimating the LC50, using the arithmetic mean of the highest concentration with total survival and the lowest of total mortality (Bliss, 1967).

6.3: Results

6.3.1: Experiment 1 - Preliminary *Daphnia magna* Exhaust-Polluted Water (EPW) LC50

In the preliminary *Daphnia magna* LC50 test, low concentrations of the artificially produced outboard motor EPW caused mortality. When expressed as the ratio of fuel:oil mixture used by the engine (to produce the contaminated water) and the amount of dilution water, the LC50 was found to be 442 ppm (v/v).

6.3.2: Experiment 2 - *Daphnia magna* LC50s, with HC Measurement

Table 6.6 shows all of the EPW LC50s in the current study from Experiment 2. In all three tests, 24 hour LC50s were calculated but unfortunately, due to control mortality exceeding 10 % after 48 hours, two out of the three tests were invalid for calculation of 48 hour LC50s. In one of these tests, five out of thirty animals died (16.7 % control mortality) while eight out of thirty (26.7 %) died in the other test. The only valid 48 hr LC50 test was that in which only two replicate dilutions of the EPW were tested and even here, there was 10 % mortality. This precluded any statistical measure of the difference between the toxicity of EPW after 24 and 48 hours, but it was found that the 48 hour LC50 was only about half of the 24 hour LC50 in this test. An indication of the variation between the end points of each individual replicate is gained from the second measure of 95 % C.I. As can be seen, low concentrations of volatile aromatic HCs caused mortality.

Table 6.6: EPW LC50s, from Experiment 2, measured as aqueous volatile aromatic HC concentrations in $mg.l.^{-1}$. 24 hr LC50s are given for all tests and 48 hr LC50s when possible. Two measures of 95 % C.I. are given: (i) for each complete test, calculated by maximum likelihood estimation using probit analysis (ii) an indication of the variability of each test is given by the second measure, which is gained from the individual replicate LC50s.

Test	*Number of organisms*	*Number of replicates*	*Mean LC50, $mg.l^{-1}$*	*Estimated maximum likelihood 95 % C.I.*	*95 % C.I. calculated from individual replicate LC50s*
1 (24 hr LC50)	20	2	2.21	2.15 – 2.27	-
1(48 hr LC50)	20	2	1.15	1.10 – 1.19	-
2 (24 hr LC50)	30	3	4.39	4.32 – 4.45	3.87 – 4.97
2(48 hr LC50)	30	3	-	-	-
3 (24 hr LC50)	30	3	3.94	3.90 – 3.99	3.44 – 4.58
3(48 hr LC50)	30	3	-	-	-

6.3.3: Experiment 3 - Water-Accommodated Fraction (WAF)

Table 6.7 shows all fuel:oil mixture WAF LC50s in the current study, including 95 % Confidence Intervals (C.I.), with all results expressed in aqueous volatile (monocyclic) aromatic hydrocarbon (HC) concentrations, as previously done for the EPW tests (see Section 6.3.2). As can be seen, the WAF of fuel:oil mixture (50:1), was less toxic than the mixture of HCs in EPW and the difference was found to be highly statistically significant, when the LC50s of the individual replicates (six WAF tests and eight EPW tests) were compared ($p = 0.0024$, $U = 69.0$, Mann-Whitney U Test).

Comparison of the 24 and 48 hour WAF LC50 results (calculated from each individual replicate) showed that there was no statistically significant difference between them ($t < 0.01$; 9 d. f.; n.s.).

Table 6.7: 24 hr and 48 hr WAF LC50s (from Experiment 3) measured as aqueous volatile aromatic HC concentrations in $mg.l.^{-1}$. Two measures of 95 % C.I. are given: (i) for each complete test, calculated by maximum likelihood estimation using probit analysis (ii) an indication of the variability of each test is given by the second measure, which is gained from the individual replicate LC50s.

Test	*Number of organisms*	*Number of replicates*	*Mean LC50, $mg.l^{-1}$*	*Estimated maximum likelihood 95 % C.I.*	*95 % C.I. calculated from individual replicate LC50s*
1 (24 hr LC50)	30	3	9.79	9.75 – 9.83	8.57 – 12.12
1 (48 hr LC50)	30	3	9.79	9.75 – 9.83	8.57 – 12.12
2 (24 hr LC50)	30	3	9.33	9.32 – 9.35	7.69 – 11.12
2 (48 hr LC50)	30	3	9.35	9.33 – 9.35	9.01 – 9.78

6.3.4: Experiment 4 - *Diaptomus gracilis* LC50

Outboard motor EPW was highly toxic to *Diaptomus gracilis* as well as *Daphnia magna*. The results of the *D. magna* LC50 tests in Section 6.3.1 and the *D. gracilis* tests described here (in which the same method of EPW production was used) indicate that *Diaptomus gracilis* may be the more sensitive of the two organisms, with lower LC50s when expressed as the ratio of fuel used by the engine and dilution water (see Table 6.8). However, this difference in toxicity was not statistically significant ($U = 17.0$, n. s., $n = 3$ and 5, Mann-Whitney U-Test).

6.3.5: Experiment 5 – Recovery of Water Quality, using *D. gracilis* LC50s

Based on the results of a subsequent LC50 test using *Diaptomus*, recovery of water quality was shown to have taken place after the EPW in the tank was allowed to remain undisturbed for 5 days. The toxicity of EPW was significantly lower after this 5 day period than it was immediately after engine operation (Mann-Whitney U = 15.0, p = 0.0047, n = 5 for each data set; see Table 6.8).

Further recovery of water quality was evident 43 days after engine operation (see Table 6.8). Indeed, it was not even possible to precisely calculate the LC50 on 43 day old EPW – only give a minimum value. No test organisms died, even at the highest EPW concentration (i.e. 100% concentrated 43 day old EPW), indicating that the toxicity of the 43 day old EPW had decreased over this time period, to levels undetectable in the *D. gracilis* LC50 test. These ecotoxicological experiments give evidence that water quality does recover, following powerboat exhaust pollution, further confirming the results of the chemical experiments described in Chapter 5, section 5.4.6.

Table 6.8: *Diaptomus gracilis* LC50 results: comparison with *Daphnia magna* and investigation of recovery of water quality from powerboat pollution.

Species	Test	Number of organisms	Number of replicates	24 hr. LC50 (fuel consumed/ dilution water, ppm v./v.)
Daphnia magna	Freshly produced EPW	30	3	442
Diaptomus gracilis	Freshly produced EPW	50	5	300
	5 day old EPW	50	5	1878
	43 day old EPW	50	5	>9183

6.4: Discussion

6.4.1: General

All of the 24 hour LC50 results obtained in the current study were unequivocal, with a high degree of concordance between the replicates. In these tests, both outboard motor EPW and the outboard motor fuel: oil mixture WAF were found to cause test organism mortality.

Control survival was greater than 90 % in all of the 24 hr tests. This fulfils the criteria in OECD (1984) and Cooney (1993).

There were negligible HC concentrations in the control vessels after testing. This indicates:

a) that the sample purification process (Table 4.3) successfully removed any biogenic material.
b) very little cross contamination occurred during toxicity tests.
c) all glass cleaning procedures were satisfactory.

In some of the EPW LC50 tests in the current study, control mortality exceeded 10 % after the duration of 48 hours, invalidating the 48 hour LC50 results (Cooney, 1993). In each test, the test chambers needed to be dismantled in order to determine the number of affected organisms after 24 hours. It is possible that the reassembly of these devices, after measuring the mortality after 24 hours may have caused stress to the organisms and may explain the high control mortality in some of the 48 hour tests.

As yet, there has only been one study (Dean and DeGraeve, 1986) in which such screen devices were used. Future research would be useful, both involving production of better screen devices (that would not harm the Daphnids) and also in developing better methods in general, for the assessment of the toxicity of volatile, poorly soluble, complex mixtures (Baird, 1995).

6.4.2: Lethal Toxicity of EPW to *Daphnia magna*

6.4.2.1: Assessment of the Toxicity of Outboard Motor Exhaust Pollutants

Using *Daphnia magna* as a test organism, the mean LC50 for outboard motor Exhaust-Polluted Water (EPW), was 3.72 $mg.l^{-1}$, expressed as the total aqueous concentration of volatile aromatic HCs. This approximates to a total concentration of aqueous organic compounds of 4.10 $mg.l^{-1}$ (including PAHs and oxygenated HC derivatives). This estimate is based on a previous study by Jüttner *et al.* (1995a) in which the principal organic compounds in two-stroke outboard motor EPW were quantified. It was found that PAHs and oxygenated compounds formed 9.3 % of the total aqueous concentration of these compounds.

The toxicity of outboard motor EPW determined in the current study is relatively high, compared with many other substances which have been tested in previous studies. In a comprehensive study of the effects of a wide range of water pollutants, Kuhn *et al.* (1989*b*) found LC50s for 79 different compounds. The mixture of organic compounds in EPW was found to be more toxic, weight for weight than 56 of these.

Table 6.9: Comparison of the toxicity to *Daphnia magna* of EPW and fuel:oil mixture WAF with that of some of the more prominent components of both mixtures. Data from Kuhn *et al.* (1989*b*) and the present study.

Author	Test Substance	LC50, $mg\ l^{-1}$
Kuhn *et al.*, 1989*a*	Methyl benzene (Toluene)	84.00
	1,3,5 Trimethylbenzene (Mesitylene)	50.00
	4 methyl phenol (p-cresol)	4.90
Bannan, 1999	EPW (expressed as the concentration of volatile aromatic HCs)	3.72
	EPW (expressed as the estimated total concentration of organic compounds)	4.10
	Fuel:Oil Mixture WAF	9.88

Table 6.9 shows the toxicity to *Daphnia magna*, of three different pure HC compounds, which are found in EPW (data from Kuhn *et al.*, 1989*b*), and compares these to the mixture of organic chemicals in EPW, measured in the current study and expressed as the total concentration of volatile aromatic HCs. The LC50 of the WAF of the fuel:oil mixture in the current study is also shown.

From this data, it is apparent that the mixture of compounds found in EPW is likely to be more toxic than most of its major individual components. This is probably due to the higher toxicity of some compounds, particularly PAHs and phenolic compounds, both of which are only present in low concentrations in EPW but may be contributing a relatively high proportion of its toxicity.

PAHs are particularly toxic and in a study by Gala and Giesy (1992), the toxicity of anthracene (a PAH, found in EPW in the current study - see Ch. 3) to the growth rate of *Selenastrum capricornutum* was investigated. A 24 hr. EC50 of 3.3 μg l^{-1} was calculated and it was also stated that invertebrates and fish were even more sensitive than this.

6.4.2.2: Comparison with Previous Studies

Some previous studies on the toxicity of exhaust-polluted water have expressed aqueous petroleum HC concentrations as the ratio of the volume of fuel consumed by the engine to the volume of dilution water. This method of quantification allows comparisons to be made between studies in which no other method of quantification was available, as is the case with many older studies (including the earlier experiments in the current study) in which hydrocarbons in the polluted water were not directly quantified. Table 6.10 compares a number of studies (including the present study) in this manner.

As can be seen from the results presented in Table 6.10, there is some variation in the LC50 end points of each test. This could be due to both different sensitivities to HCs of the different organisms and also maybe the older engines used in the previous studies, which may have had higher HC emission rates. Despite these differences, the results are comparable.

Table 6.10: Comparison of the results of the LC50 tests in the present study with those of previous studies.

Study	Test Organisms	24 hr. LC50 (fuel consumed/ dilution water, ppm v./v.)
English *et al.* (1963)	Fathead Minnows (*Pimephales promelas*)	566
	Bluegills (*Lepomis macrochirus*)	689
Kuzminski *et al.* (1972)	Fathead Minnows (collected in spring)	167
	Fathead Minnows (collected in summer)	465
	Bluegills ¾-1"	320
	Bluegills 2-2½"	440
Preliminary Experiments in The Current Study	*Daphnia magna*	442
	Diaptomus gracilis	300
Later, More Precise Experiments in The Current Study	*Daphnia magna*	336

6.4.2.3: Consideration of Indirect Toxicity of EPW

It is unlikely that *Daphnia* were adversely affected by indirect toxicity in EPW tests, due to agents not present in the WAF tests. These include:

- low water hardness.
- differences in pH.
- oxygen deficiency.
- Carbon Monoxide (CO).

It is not likely that low water hardness affected the results of the EPW tests in the current study. The main evidence for this is seen in the acclimatisation process of adult *Daphnia* to Loch Lomond water, which was successful. The fecundity of these adults was apparently unaffected by the transfer process, with just as many neonates produced after the transfer to Loch Lomond water. Since Loch Lomond water had no effect on the reproduction rate of *Daphnia* in culture, it is highly unlikely that any mortality could have resulted from neonates being placed in Loch Lomond water in the actual tests.

Changes in pH in toxicity testing can usually be explained by changes in water hardness (Rattner and Heath, 1995). Thus, since hardness clearly did not affect the results of any of these tests, it was not felt to be necessary to monitor pH levels in the toxicity test media in the present study.

More measurements of dissolved oxygen in the test media would have been desirable, but there was only limited access to a suitable oxygen electrode was possible. However, the few available results were unequivocal, with the lowest O_2 concentration (86.8 %) being well in excess of the 60 % minimum criterion recommended by the OECD (1984). This suggests that lack of oxygen was not a factor causing adverse effects to *Daphnia.*

There is also a hypothesis that indirect toxicity due to Carbon monoxide (CO) in EPW (absent in WAF) occurred. A number of previous studies have discounted this, claiming that CO is an air pollutant (Jackivicz and Kuzminski, 1973; BIA, 1975; East Midlands Regional Council for Sport and Recreation, 1979; Butcher, 1982). It must be borne in mind that little research has been carried out on this subject yet and future work could still be useful. In one study (Kempinger *et al.*, 1998), CO was claimed to have killed fish near an outboard motor testing unit. However, in that study, HCs were completely ignored and future work is necessary to evaluate the relative contribution of each substance to the toxicity of powerboat EPW.

6.4.3: Water-Accommodated Fraction (WAF) LC50s

The WAF in the current study was found to be fairly toxic (see Table 6.7). When its toxicity is compared to that of a wide variety of different pure compounds, the LC50 measurements showed that the WAF (expressed as the total concentration of volatile aromatic HCs) was more toxic than 44 out of the 78 compounds tested by Kuhn *et al.* (1989). However, the toxicity was statistically significantly lower than that of the EPW tested in the current study and there could be two reasons for this:

- Some oxygenated organic compounds, particularly phenols, but also including benzaldehyde and formaldehyde are absent in fuel:oil mixture but present in exhaust-polluted water (Jüttner 1994; Jüttner *et al.*, 1995*a*). These partially oxidised compounds are more toxic than their corresponding parent (Kuhn *et al.*, 1989*b*) hydrocarbon compounds and contribute significantly to the toxicity of EPW.
- The highly toxic PAH compounds are likely to be relatively more abundant in EPW than in the WAF (see Chapter 3), as the more volatile compounds in fuel evaporate more quickly than the PAH, following the combustion process. This relative increase in PAH could also be contributing to the enhanced toxicity of EPW.

In spite of the high proportion of the toxicity of EPW attributable to oxygenated compounds and PAHs, the relatively high toxicity of the WAF suggests that volatile aromatic HCs are indeed toxic and are also responsible for nearly half of the toxicity of EPW.

It was deemed to be less necessary to validate dilution process for WAF than EPW, as it was felt that the former was a more homogenous substance. This is apparently confirmed by the variation of the LC50 results between replicates being greater for EPW, although more research would be useful.

6.4.4: Losses of Volatile Aromatic HCs in Toxicity Tests

The volatile aromatic HCs in EPW and WAF evaporated very quickly in the vessels used for toxicity testing, posing a problem in the calculation of the LC50s. The situation is further complicated by different evaporation rates of different hydrocarbon (HC) compounds (see Weast, 1988) and the high volatility and poor water solubility of most HC compounds. In the current study, the best way of dealing with this was thought to be the expression of the LC50 end points using the initial HC concentrations before testing This gives a conservative estimate of HC toxicity, as a higher LC50 is gained than that using mean HC concentrations throughout the test. It is likely that most of the deaths of the organisms tested could have taken place in the first few hours following commencement of the tests, while HC concentrations were still relatively high.

There is also evidence for a high rate of losses of HCs from water in previous work on the toxicity of outboard motor exhaust. In one study (English *et al.*, 1963), lethal toxicity of outboard motor EPW to fathead minnows (*Pimephales promelas*) was calculated and it was found that no fish were killed after 96 hours additional to those already dead after 24 hours. Flow through tests were also carried out, and in this case, only a small proportion of additional fish were killed during the second to fourth days of 96 hour LC50 tests. Further related work, possibly using flow through tests for *Daphnia magna* coupled with direct measurement of aqueous HCs, would be useful.

Current standard LC50 test conditions are suited to pure compounds, of low volatility and high water solubility, a typical example being sodium bromide (NaBr) which is used as a reference toxicant to *Daphnia magna* (Baird *et al.*, 1989*a*). There has been some work on the toxicity testing of volatile and poorly water-soluble compounds, with previous studies having made use of closed vessels in the case of volatile compounds and solubilisers in the case of poorly soluble compounds (Cooney, 1993; Juchelka and Snell, 1995). The use of either of these devices in the current study could have caused additional difficulties. Closed vessels could have

caused depletion of oxygen and solubilisers were unnecessary, as the compounds in outboard motor EPW were themselves partially water-soluble. It may be useful to carry out LC50 tests on *D. magna*, with EPW and WAF, using closed vessels, to:

1) see if HC evaporation can be minimised.
2) see if lack of oxygen occurs and if so, to refine the method appropriately.
3) compare the toxicity of the aforementioned substances in closed and open vessels.

6.4.5: *Diaptomus gracilis* LC50 Tests

Some LC50 tests were carried out using *Diaptomus gracilis*, the results of which suggest that this organism could be more sensitive to outboard motor pollution than *Daphnia magna*. However, this is not certain for a number of reasons:

1) HCs were not measured in the *D. gracilis* tests.
2) The test organisms were taken from natural populations in Loch Lomond, for which there could be sensitivity differences to EPW HC pollutants at different times of the year.
3) the test was not replicated.

To investigate potential differences in sensitivity to powerboat pollutants, LC50 tests could be carried out at different times of the year. In addition, better replication of tests, involving the measurement of HCs in the test media is essential in any future work.

Future basic research would also be useful, involving the testing of *Diaptomus gracilis* to a range of representative reference toxicants, to confirm whether it is an appropriate aquatic toxicity testing organism. This would also help to find optimum conditions for LC50 testing. In the current study, test conditions were simply modelled on standard conditions for *Daphnia magna*, with few adjustments. It is possible that such conditions may not have been optimal for *Diaptomus*.

6.4.6: Theoretical Safe Levels of Powerboat Exhaust HCs in the Aquatic Environment

Currently there are no recognized standard methods for the estimation of safe levels of complex mixtures in the aquatic environment. In the present study, three procedures were used, each based on existing methods for the derivation of approximate safe levels of single compounds, which are comparatively well established.

The first method involved the assumption that the mixture of HC compounds in EPW could be treated as a single compound with a toxicity equivalent to the sum of the toxicities of the mixture's individual compounds. For single compounds, the OECD (1992) has estimated "environmental concern levels" of a given pure compound to be 1000 times less than the LC50. Approximate safe levels of the total concentration of volatile aromatic HCs in outboard motor EPW were gained in this manner and found to be 3.72 $\mu g.l^{-1}$, expressed as the total concentration of volatile aromatic HCs.

The second method involved a similar approach to the first, using the LC50 data from the WAF tests, and thus deriving a theoretical safe level of volatile aromatic HCs. This was calculated as 9.88 $\mu g.l^{-1}$.

Table 6.11 shows the sites in Loch Lomond at which approximate safe levels (based both on EPW and WAF LC50s) of powerboat exhaust pollutants were exceeded, in the summer of 1996. As can be seen, the safe levels based on EPW toxicity tests were exceeded in both surface and sub-surface water at many of the sampling sites on Loch Lomond in the summer of 1996. Even using a more conservative approach and WAF toxicity test data, (consequently higher) arbitrary safe levels were still exceeded in some of the samples.

Table 6.11: Actual concentrations in (mostly surface) water samples from Loch Lomond during the summer of 1996, compared to estimated safe levels, calculated on the basis of LC50 tests in the current study. The sites at which these approximate safe levels are exceeded and the local density of powered craft are given. Unless indicated by this symbol (>1), all sites were sampled only on July 21st 1996. When more than one sample was taken, the highest concentration is indicated, and the boat density on that occasion.

Site	Powerboat Density (boats km^{-2})	Exhaust pollutant levels ($\mu g\ l^{-1}$)	Sites exceeding safe levels based on EPW LC50 tests	Sites exceeding safe levels based on WAF LC50 tests
Field Station Bay	6.05	3.02		
Field Station Bay, sub-surface	6.05	2.70		
Mid-Basin Reference Site (>1)	6.05	4.14	*	
Mid-Basin Reference Site, sub-surface (>1)	6.05	2.40		
Milarrochy Bay Outer	22.98	6.59	*	
Milarrochy Bay Inner	74.08	33.56	*	*
Balmaha Outer Moorings	2.25	1.68		
Balmaha Pier (>1)	1.96	10.60	*	*
Area 3 Reference Site	5.59	5.49	*	
Area 3 Reference Site, sub-surface	5.59	3.73	*	
Cameron House	5.59	6.75	*	
River Leven Exit (>1)	5.59	6.15	*	
Area 4 Reference Site	3.46	1.98		
Area 4 Reference Site, sub-surface	3.46	3.52		
Inchmoan S. Beach	3.46	6.75	*	
Haven	46.83	4.78	*	
Haven, sub-surface	46.83	5.69	*	
Skiers Passage???	46.83	7.06	*	
Narrows Outer	46.83	9.00	*	
Narrows Middle	46.83	12.18	*	*
Narrows Inner (>1)	46.83	36.55	*	*
Narrows Inner (>1), sub-surface	46.83	10.01	*	*
Luss Outer	6.05	10.32	*	*
Luss Inner	113.89	33.56	*	*
Inverbeg Moorings	4.09	6.21	*	
Area 10 Reference Site	1.73	4.42	*	
Area 10 Reference Site, sub-surface	1.73	1.97		
Area 12 Reference Site	7.26	6.52	*	
Area 12 Reference Site, sub-surface	7.26	5.82	*	
Ardlui Marina	7.26	18.31	*	*

The third method for the assessment of the risk of powerboat HC pollution in Loch Lomond used actual EC guidelines for safe levels of two single compounds (Benzene and Toluene) and one mixture of three pure compounds (1,2-, 1,3- and 1,4-dimethyl benzene). The Commission of the European Community (CEC, 1994*b*) assessed the environmental risk of these substances and calculated safe levels of 10 $\mu g.l^{-1}$ for each. This method had only a limited application to the current study, as the levels of these particular compounds were only measured (using GC-MS: see Ch. 3) in eight of the 105 samples taken from the loch during the summer of 1996 (see Ch. 4, Section 4.3.8).

All of the 1996 samples analysed by GC-MS had relatively low overall concentrations of volatile aromatic HCs. In none of these samples were the concentrations of either benzene or the mixture of dimethylbenzenes in excess of 10 mg.l-1. Toluene could not be analysed at that time, due to atmospheric contamination of the laboratory (see Appendix V).

The hydrocarbons in outboard motor Exhaust-Polluted Water (EPW) constitute a complex mixture, but its compounds have very similar chemical characteristics. In a study by Doi (1993), the toxicity of different types of complex mixtures was examined. In most cases, toxicity is additive and this mechanism has been found to be particularly characteristic of organic compounds. Interaction between these compounds is very unlikely, consequently making either synergism or antagonism unlikely. This lack of interaction indicates that the toxicity of the different compounds in EPW should also follow an additive mechanism, whereby the toxicity of the mixture is equal to the sum of the toxicity of its individual compounds. The most toxic compounds in EPW, including PAHs, therefore contribute more to the overall toxicity. It may thus be appropriate in this case to treat EPW as one hypothetical hydrocarbon (HC) "compound", with a measured lethal toxicity equal to that of the mixture of HCs in EPW. For the purposes of environmental risk assessment, this would suggest that the use of single compound application factors was justified, in the derivation of approximate safe levels.

It must be borne in mind that the sites from which water samples were deliberately chosen in areas of high boat activity, and the HC concentrations in the reference sites (with low levels of boat activity and representative of most of the area of Loch Lomond; see Ch. 5, Section 5.4.2) only exceeded safe levels in 4 out of 25 samples, even when basing safe levels on the results of EPW toxicity tests. In addition, high boat activity generally only occurs on summer weekend days with favourable weather conditions (see Adams *et al.*, 1992).

It would be useful to examine the sensitivity of other local species, including algae and fish. The OECD (1992) recommend lower safety application factors (100) in the case of known data on acute toxicity to at least one species of these three taxa, and such data would improve the accuracy of any estimation of environmentally safe concentrations of EPW pollutants (although not to the same extent as good quality chronic toxicity data).

6.4.7: Limitations of the Toxicity Testing Procedures in the Current Study

Due to an oversight, it was only found out after all toxicity tests were completed that "Thames" leaded petrol was used in the preliminary (1994) experiments and that "Thames" unleaded petrol was used in the later more precise studies (in 1997). However, the aquatic toxicity of EPW produced by leaded petrol is very unlikely to be significantly different to that produced by unleaded. Firstly, the proportion of fuel used that is exhausted to the environment is unlikely to differ. Estimates of this have varied, but show no pattern. Earlier calculations were produced at times when leaded petrol was in almost universal use and Jackivicz and Kuzminski (1973) stated that from 10 to 16 % of the HC in outboard motor fuel are exhausted to the environment. More recent estimates have taken place when unleaded fuel had almost completely superseded leaded fuel and vary from 8.3 %, calculated by Coates and Lassanske (1990) to 25 % (US EPA, 1991).

The toxicity of lead to *Daphnia magna* was found in a study by Khangarot and Ray (1989) and an LC50 of 3.61 $mg.l^{-1}$ was gained. The amount of lead in polluted water was not measured in the earlier part of the present study, but it can be estimated. Previous studies (see Jackivicz and Kuzminski, 1973) have shown that approximately 22 % of the lead in leaded petrol reaches the water when consumed by an outboard motor. Modern leaded petrol sold in the UK today has a mean lead content of 0.15 $g.l^{-1}$ (Pearce, 1996). Using these measurements, the lead content of the EPW in the earlier studies can be estimated as 0.24 $mg.l^{-1}$. A dilution of the EPW of 5.51 % was found to be lethal to *D. magna*, and the lead concentration of the medium at this EPW dilution would be only about 13 $\mu g.l^{-1}$, a level 278 times lower than the expected lethal concentration. As any toxic interaction between hydrocarbons and any other substances is unlikely (Doi, 1993), such concentrations of lead would be very unlikely to contribute to the acute toxicity of EPW. Further research could help to confirm this.

6.4.8: Further Research

6.4.8.1: Toxicity of Hydrocarbons

There is tremendous scope for further relevant investigations of the ecotoxicology of hydrocarbons (HCs), with particular relevance to Loch Lomond. Powerboat hydrocarbon (HC) pollution not only emanates from petrol, but also lubricant oil and diesel. Outboard motors emit lubricant oil in addition to petrol, and it would be very useful to compare the toxicity (both acute and chronic) of water-accommodated fractions (WAF) of pure petrol, pure lubricant oil and fuel:oil mixtures.

So far, there has only been one study comparing the toxicity of diesel to that of petrol to freshwater organisms. Gaur and Singh (1989) found that diesel was twice as toxic as petrol to the alga *Anabaena doliolum*. The high toxicity of diesel could be important in the context of Loch Lomond, as many boats are powered by diesel engines. Although the annual total of HC emission from these diesel powered boats is

low compared to petrol-powered outboard motors (Ch. 2, Section 2.3.4), the higher toxicity of diesel and also the spillage of diesel that occurs (see Ch. 5, Section 5.5.4 and Appendix VII) represents a potential threat to the environment of Loch Lomond. Further toxicity testing with *Daphnia* could be very useful, both using diesel engine exhaust polluted water (EPW) and diesel WAFs.

One feature of all WAFs is their relative ease of production compared to EPW. This would allow good replication of experiments and consequently more accurate calculations of HC toxicity in future studies.

6.4.8.2: Method Development of Toxicity Tests

Other further work of a more general nature is also required, in order to better understand the toxicity of "difficult" substances i.e. complex mixtures, poorly soluble and volatile substances (Baird, 1995) both in laboratory situations and in the aquatic environment. More accurate estimates of safe levels of complex mixtures would then be possible. There has been a lack of research in the formulation of standard methods of assessment of the toxicity of such substances, but recommendations for such toxicity tests have been given in a recent study by Rufli *et al.* (1998).

One useful method of approach in future studies is the production of microcosms. There has been little research on HC pollution of freshwaters using this approach, with only one study so far using microcosms to assess the toxicity of diesel oil to freshwater organisms (Johnson and Romanenko, 1989). It was found that these tests were very sensitive, with diesel having gross adverse effects on the biota of these microcosms at concentrations of only 1 $mg.l^{-1}$.

6.4.8.3: Bioaccumulation

Many of the HCs in EPW degrade relatively quickly in the aquatic environment, although some of them, such as PAHs are more persistent and may bioaccumulate. This is a well known phenomenon in marine ecosystems, particularly for filter feeding bivalve molluscs and has been demonstrated in a number of studies (McDonald *et al.*, 1992; Hellou *et al.*, 1993, 1994; Shchekaturina *et al.* 1995). Bioaccumulation can also occur in a variety of ways at different trophic levels in freshwater ecosystems. Southworth *et al.* (1978) demonstrated that *Daphnia pulex* can directly absorb PAHs from water to body lipids. This occurs to the greatest extent in the case of heavier PAHs with poorer water solubility and higher toxicity (such as benz-(a)-anthracene ($C_{18}H_{12}$). Van der Oost *et al.* (1994) showed that the situation is more complicated in freshwater fish. Unlike *D. pulex*, fish do not accumulate PAHs by simple absorptive uptake alone (although this does occur to some extent). PAHs from ingested food can produce toxic metabolites and DNA adducts in the liver. The characteristics of PAH bioaccumulation in fish contrast with those of chlorinated organic compounds, such as pesticides and their derivatives. The latter exhibit the well known phenomenon of biomagnification (absent in PAHs), whereby the levels of the compounds increase with increasing trophic level (Spacie *et al.*, 1993).

The present study could be usefully complemented by investigating chronic toxicity that may be arising from PAH bioaccumulation in the living organisms of Loch Lomond. Both Daphnids (including *Daphnia pulex*) and fish, such as pike (*Esox lucius*) and eels (*Anguilla anguilla*) could be investigated. Preliminary investigations would be useful, to:

1) see if PAHs are present in significant concentrations in water and sediment of Loch Lomond.
2) ascertain the source of PAHs in Loch Lomond.

Chapter 7

General Discussion and Conclusions

7.1: Summary and Review of Results

7.1.1: Overall Conclusion

The results of the current study justify concern expressed about potential adverse effects of powerboat hydrocarbon (HC) emissions on the water chemistry and zooplankton communities of Loch Lomond. In summer, at times and locations of high powerboat activity, theoretical environmental safe levels of powerboat exhaust hydrocarbons (HC) in the water can be exceeded. This is the case, even when using three different methods to derive these safe levels.

The only other known study, in which a comprehensive risk assessment of powerboat (particularly outboard motor) activity on the aquatic environment was carried out, was that of the Boating Industry Associations (BIA) (1975). In that study, it was concluded that powerboats posed no measurable risk to the aquatic environment, but this conclusion has little relevance nowadays, due to improvements in analytical methods and a greater appreciation of risks to the environment.

7.1.2: Assessment of the Scale of Powerboat Pollution in Loch Lomond

A preliminary assessment of the risk of powerboat pollution in Loch Lomond was conducted, by estimating the loch's total annual HC input from powerboat emissions (Ch. 2). It was possible to achieve this by using long-term data on craft activity on Loch Lomond, published information on engine HC discharge and some assumptions of craft activity. It was found that over 25 tonnes of HC material was emitted into the loch in 1989, with the vast majority of this emanating from speedboats, propelled by two-stroke outboard motors. Boat census data from 1989 to 1997 have shown that the annual HC discharge has increased dramatically over this time period. More research needs to be carried out, particularly into the extent of fuel spillage, which is not included in this model.

7.1.3: Water Chemistry Changes Following Powerboat Operation

In the current study, water quality changes caused by powerboat operation were investigated by running an outboard motor in an experimental tank under controlled conditions and analysing the pollutants by Gas Chromatography-Mass Spectrometry (GC-MS; see Ch. 3). The main constituents of Exhaust-Polluted Water (EPW) collected immediately following engine operation were:

- Benzene
- Methyl benzene (toluene)
- Dimethyl benzenes
- Other alkylated benzenes
- Polycyclic Aromatic Hydrocarbons (PAHs)
- Phenolic compounds
- Benzaldehyde compounds

The latter two groups of compounds are partially oxidised hydrocarbons produced by combustion and not found in raw fuel.

7.1.4: An Alternative Method for the Analysis of Powerboat Exhaust HC in Water

In the present study, an alternative analytical method (to GC-MS) for the detection and quantification of volatile aromatic HCs was successfully developed (Ch. 4). This method involved the adaptation of existing techniques for analysis of similar HCs by fluorescence spectroscopy and it had a number of important attributes:

- it was very fast, allowing the analysis of hitherto unfeasible numbers of water samples.
- calibration of the spectrofluorimeter was relatively straightforward, and precision of measurement was good.
- the method was specific to volatile aromatic HCs.
- the method was highly sensitive with detection limits of 0.4 $\mu g.l^{-1}$ in freshwater.
- the method was non-destructive, allowing secondary analyses.

The modified method facilitated a series of laboratory and field experiments investigating:

1) the spatial distribution of powerboat exhaust HCs in Loch Lomond.
2) the depth profile of powerboat exhaust HCs.
3) the recovery of water quality following powerboat exhaust pollution.

7.1.5: The Spatial Distribution of Powerboat Exhaust HCs in Loch Lomond

A large-scale survey was carried out on Loch Lomond, on one weekend day in the summer of 1996 (Ch. 5, Section 5.3.2.2). It was found that there was a positive correlation between local boat densities and HC concentrations in surface waters. There was some unpredictability in this relationship, possibly due to differences in weather conditions and known differences in boat distribution patterns between Saturdays, Sundays and different holiday types (Brown and Adams, 1995). It was also found that the contribution of boat fuel spillage to HC contamination at some sites on Loch Lomond could be significant. By contrast, road runoff and atmospheric deposition are very unlikely to explain the origin of the volatile aromatic HCs detected in Loch Lomond in summer.

Very similar conclusions to those of the present study were gained in a study by Miller and Fiore (1997), in which it was found that boat activity raised concentrations of volatile aromatic HCs in Lake Tahoe, California, USA.

There is great scope for conducting future studies on powerboat pollution, particularly by diesel and two-stroke lubricant oils, both of which could be significant contaminants of many freshwater ecosystems, including Loch Lomond. Only minor refinements of the methods (fluorescence spectroscopy and GC-MS) used in the present study would be necessary for this, as some of the components of diesel and two-stroke lubricant oils (like all petroleum oils) fluoresce, but at different wavelengths to the volatile (monocyclics: i.e. benzene and its derivatives) aromatic HCs from petrol.

7.1.6: The Depth Profile of Powerboat Exhaust HC

In the present study, it was comprehensively proven by laboratory and field experiments that volatile aromatic HCs from powerboat exhausts are not confined to the surface microlayer (the top 50 μm, according to Hardy *et al.*, 1990) of the water column, instead being found at depths of at least 1.5 metres in Loch Lomond (Ch. 5, Section 5.3.2.4). This agrees well with the only previous related study (Miller and Fiore, 1997), in which powerboat-derived volatile aromatic HCs were found at depths of at least 4 metres in Lake Tahoe, California, USA. Further research would be useful, to see if powerboat exhaust HC pollutants are found at even greater depths.

7.1.7: The Recovery of Water Quality after Powerboat Exhaust Pollution

Recovery of water quality following pollution by an outboard motor was demonstrated in the present study in a series of three controlled experiments, carried out in an experimental tank (Ch. 5, Section 5.4.6.2). Half-lives for the losses of volatile aromatic HC from the water were ascertained, with a mean of 8.7 days calculated.

Recovery of water quality in the field was not evident, possibly due to input of volatile aromatic HCs (possibly from boat fuel spillage) occurring at unexpected times (Ch. 5, Section 5.4.6.1). Recovery obviously does occur, at least in the longer term, because volatile aromatic HCs were found to be undetectable in winter at a number of sites in the loch, including two with high summer powerboat activity. Further research could be useful, involving sampling throughout the course of a year, in order to investigate the longer term recovery of water quality. In the short-term, recovery of water quality following pollution episodes in the field may be affected by wind and water currents and this could be investigated in controlled experiments involving artificially-induced agitation of the water.

7.1.8: The Toxicity of Powerboat Exhaust Polluted Water.

Using *Daphnia magna*, the toxicity of outboard motor EPW (expressed as aqueous volatile aromatic HC concentration) was found to be relatively high (Ch. 6, Section 6.4.2.1). The toxicity of EPW was also found to be significantly higher than that of a water soluble fraction (WSF) of unburned petrol:oil mixture, with measurements expressed as the aqueous concentration of organic compounds in both cases.

There is great scope for future research in the toxicity of all powerboat exhaust and fuel components, and this could utilise a number of different approaches:

1) The toxicity of WSFs of pure petrol, pure lubricant oil and diesel oil could be investigated. This approach has the great advantage of ease of experimental preparation, as WSFs of petroleum oils are a lot easier to prepare than EPW. Better replication of experiments and consequent greater accuracy would then be feasible. The ease of preparation of these WSFs would also facilitate the conduction of chronic toxicity tests.
2) So far, no investigations have been carried out on pollution by diesel powered craft. Although the total input of HC emissions from diesel powered craft is likely to be lower than that from petrol driven boats, there is evidence that spillage of diesel could be significant in Loch Lomond (see Appendix VII). In addition to this, weight for weight, diesel has been shown to be more toxic than petrol to aquatic organisms, by means of algal toxicity tests (Gaur and Singh, 1989).
3) Toxicity testing of polluted sediment could also be a useful approach in future studies. The toxicity of contaminants in sediment to aquatic organisms is not as well understood as that of contaminants in water (Ingersoll, 1993). In order to ascertain the toxicity of HC-polluted sediment (either by petrol, diesel or lubricant oil), preliminary controlled experiments would be necessary, to examine HC partitioning between the water and the sediment and their behaviour (including degradation and transformation) in the sediment.

7.1.9: Safe Levels of Powerboat Pollutants

In the current study it was found that theoretical safe levels of powerboat exhaust pollutants were exceeded in many water samples taken from Loch Lomond, at times of high powerboat activity in summer. However, the safe levels calculated here must be viewed as approximate estimates. The accurate determination of a safe level of a pollutant depends on accurate toxicity data and such data was gained only for lethal toxicity of outboard motor EPW and a WSF of outboard motor fuel to *Daphnia magna* in the current study. Four approaches to future studies could be utilised, to improve estimates of theoretical safe levels of powerboat exhaust pollutants:

1) Toxicity tests could be performed using freshwater algae and fish, in addition to crustaceans. The OECD (1992) has stated guidelines for the estimation of safe levels of pollutants when toxicity test data is available for at least one species of each of the aforementioned taxa. The lowest lethal concentration of the three taxa is multiplied by an application factor of 0.01 to derive such safe levels.
2) Microcosm toxicity tests could also be conducted. There has not been much work using microcosms for the determination of the toxicity of petroleum HC to freshwater organisms, but in one study Johnson and Romanenko (1989) found that diesel was highly toxic, when tested with microcosms containing algae, Daphnids, chironomid larvae, sediment and water.
3) A third very different approach could involve a slight modification of the traditional method of pollution risk assessment of gauging ecosystem stress by examination of the pelagic and benthic faunal communities. In its unmodified form this is a standard approach (see Connell and Miller, 1984). A range of locations in Loch Lomond (and possibly including other lochs) could be compared with respect to their faunal communities (either pelagic or benthic). Instead of predicting HC-induced stress by boat numbers, or HC measurements in water and sediment, tissue level measurements of HCs and their metabolites could be measured and this could possibly be a predictor of potential community structure changes caused by powerboats.
4) The most accurate method to ascertain a safe level of a pollutant is to multiply a No

Observable Effect Concentration (NOEC), derived by experiment, by a suitable application factor. As the NOEC is usually much nearer to genuinely safe levels in the environment, a lower application factor, usually 0.1, has been deemed to be adequate to derive a theoretical environmentally safe level (OECD, 1992).

7.2: Further, More General Research

7.2.1: The Environmental Fate of Powerboat Exhaust Pollutants

There have been very few studies carried out so far on the environmental fate of powerboat exhaust HCs. In one study (Miller and Fiore, 1997), it was found that methyl tert-butyl ether (MTBE, an oxygenate, sometimes added to petrol to improve engine performance, particularly in the USA) could accumulate in a lake (Lake Tahoe, California, USA) following continued powerboat operation. Volatile aromatics were not found to accumulate to the same extent. Other previous work has shown that Polycyclic Aromatic Hydrocarbons (PAHs) can accumulate in sediment as a result of powerboat operation (Mastran *et al.*, 1994). One important area of future research would be to investigate the fate of all powerboat-derived contaminants, including components of petrol, diesel and lubricant oil, particularly PAHs in living organisms. There has been some previous work on this subject (Southworth *et al.*, 1978; van der Oost *et al.*, 1994) but none so far specifically investigating the impact of boat derived HCs. Both laboratory and field experiments would assist such investigations.

7.2.2: Standardisation of Toxicity Tests for Mixtures of Compounds

There is a great need for further research of a more general nature to assess the toxicity of "difficult" (volatile and poorly soluble) substances (Baird, 1995), particularly complex mixtures of compounds. As yet, few standard methods have been developed for exposing aquatic organisms to such substances. One exception is a recent study by Rufli *et al.* (1998), in which recommendations were given for relevant toxicity tests. The derivation of theoretical safe levels for "difficult" substances would consequently be facilitated with greater understanding of their toxicity.

7.3: Solutions and Proposed Future Management

Any measures which improve the fuel efficiency and emission levels of powerboats in Loch Lomond would inevitably help to reduce aqueous hydrocarbon concentrations in the loch. Such measures are more fully discussed in Chapter 2 (Section 2.4.3) and would encourage the use of cleaner engines such as gas powered and four-stroke engines which have the potential to cut emissions by over 80 %.

Legislative pressure, both local (by-laws for Loch Lomond) and national (pollution regulation in general) could help to encourage efficient boating practices. The present by-laws for regulation of boating on Loch Lomond include the designation of zones, including a 150 m zone adjacent to all shorelines and a designated area of the loch adjacent to the mouth of the Endrick Water, which have strict 5 m.p.h. speed limits. To reduce hydrocarbon pollution in all environmentally sensitive areas in the loch, this could be taken one step further, with zones created which are restricted to the use of non-powered craft only. As a consequence, pristine areas in the North Basin of Loch Lomond could benefit from this.

There has already been some research and development which has led to the production of cleaner, less polluting two-stroke engines. A special fuel injection device has been invented, which can reduce HC pollution in two-stroke engines by 75 % (Hamer, 1994). In addition to this, Jüttner *et al.* (1995*a*) describes a type of catalytic converter for use in two-stroke outboard motors, which can reduce emissions even more dramatically. Details are lacking, but future research and investment in such technology could help to reduce powerboat pollution. In addition to this, legislation could be introduced by national governments to set emission standards for powerboat engines, similar to that already in practice for automobile engines. Such legislation would compel powerboat engine manufacturers to produce cleaner outboard and inboard engines, while preventing the use of older, sometimes highly polluting motors. Furthermore, some engines, such as electric and gas powered engines, produce no aquatic HC pollution whatsoever and the use of these engines could be encouraged in environmentally sensitive areas.

References

Adams, C. E., Tippett, R., Nunn, S, and Archibald, G. (1992); The Utilisation of a Large Inland Waterway (Loch Lomond, Scotland) by Recreational Craft; *Scottish Geographical Magazine*; **108(2):** 113-118.

Adams, C. E. and Tippett, R. (1994); *Loch Lomond Recreational Craft Census, 1993*; A report commissioned by the Loch Lomond Park Authority and Scottish Natural Heritage; February 1994.

Adams, C. E. (1994); The Fish Community of Loch Lomond, Scotland: its History and Rapidly Changing Status; *Hydrobiologia*; **290:** 91-102.

Adams, C. E., Duguid, R. A., Bissett, N. and Bell, A. (1997); *The Disturbance of Fish by Recreational Craft*; A Draft Report to the Scottish Sports Council, November, 1997; Fish Biology Group, University Field Station, Rowardennan, Glasgow, G63 0AW.

Adams, C. E. and Grant A. T. (1997); *Recreational Boating on Loch Lomond*; A Commissioned Report for Scottish Natural Heritage and the Loch Lomond Park Authority, February 1997.

Adams, C. E. and Grant A. T. (1998); *Recreational Boating on Loch Lomond*; A Commissioned Report for Scottish Natural Heritage and the Loch Lomond Park Authority, February 1998.

ASTM (American Standards for Testing and Materials) (1980*a*); *Standard Practice for Identification of Waterborne Oils*; Designation D 3415-79; Book of ASTM Standards, Part 31; Philadelphia, PA.

ASTM (American Standards for Testing and Materials) (1980*b*); *Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians* (Unpublished Report E-729-80); Philadelphia, PA.

Anderson J. W., Kiesser, S. L. and Blaylock, J. W. (1980); The Cumulative Effect of Petroleum Hydrocarbons on Marine Crustaceans During Constant Exposure; *Rapp. P.-v. Reun. Cons. Int. Explor. Mer.*; **179:** 62-70.

Ashton, P. G. and Chubb, M. (1972); A Preliminary Study for Evaluating the Capacity of Waters for Recreational Boating; *Water Resources Bulletin (American Water Resources Association)*; **8(3):** 571-577.

Baird, D. J., Barber, I., Bradley, M., Calow, P. and Soares, A. M. V. M. (1989*a*); The *Daphnia* Bioassay: a Critique; *Hydrobiologia*; **188/189:** 403-406.

Baird, D. J., Soares, A. M. V. M., Girling, A., Barber, I., Bradley, M. C. and Calow, P. (1989*b*); The Long-Term Maintenance of *Daphnia magna* Straus for Use in Ecotoxicity Tests: Problems and Prospects; *Proceedings of 1st European Conference on Ecotoxicology*, Copenhagen, Denmark, October 17-19, 1988; Lokke, H., Tyle, H., and Bro Rasmussen, F. (Eds.).

Baird, D. J., Barber, I, Soares, A. M. V. M. and Calow, P. (1991); An Early Life-Stage Test with *Daphnia magna* Straus: An Alternative to the 21 day Chronic Test; *Ecotoxicology and Environmental Safety*; **22:** 1-7.

Baird, D. J. (1995); Ecotoxicological Research in the Institute of Aquaculture – "Virtual Daphnia" and Difficult Substances; *Aquaculture News*; November 1995, p. 18; Institute of Aquaculture, University of Stirling, Scotland, UK.

Bannan, M., Adams C. E. and Tippett, R. (1995); *Recreational Boating on Loch Lomond*; A Report of Recreational Boating Activity on Loch Lomond from 1989-1994, commissioned by the Loch Lomond Park Authority and Scottish Natural Heritage; March 1995.

Barber, J. and Andersson, B. (1992); Too Much of a Good Thing: Light can be Bad for Photosynthesis; *Trends Biochem. Sci.*; **17(2):** 61-66.

Bartlett, P. D. (1990); *Studies of Hydrocarbon Contamination in Alpine Lakes*; Field Studies Research Centre, Fort Popton, Angle, Pembroke, Dyfed, SA71 5AD.

Best, G. A. and Dawson, J. P. (1993); Environmental Analysis, Using Gas Chromatography; In *Gas Chromatography - a Practical Approach*, pp. 283-330; Ed. P. J. Baugh; IRL Press.

Best, G. A. and Traill, I. (1994); The Physico-Chemical Limnology of Loch Lomond; *Hydrobiologia*; **290:** 29-37.

Betton, C. I. (1993); Oils and Hydrocarbons; In *Handbook of Ecotoxicology*, pp. 244-263; Ed, P. Calow; Blackwell Scientific Publications, London.

Birmingham City Council (1989); *Water Skiing Trials at Edgbaston Reservoir*; Environmental Services Department; 3 December 1989.

Bliss, C. I. (1967); *Statistics in Biology*, pp 92-124; McGraw-Hill, New York.

Boating Industry Associations (1975); *Summary Report: Analysis of Pollution from Marine Engines and Effects on Environment*; Report reference no. PB-242-177.

Bohrer, R. N. and Lampert, W. (1988); Simultaneous Measurement of the Effect of Food Concentration on Assimilation and Respiration in *Daphnia magna* Straus; *Functional Ecology*; **2:** 463-471.

Bomboi, M. T. and Hernandez, A. (1991); Hydrocarbons in Urban Runoff: their Contribution to the Wastewaters; *Water Research*; **25(5):** 557-565.

Boxall, A. B. A. and Maltby, L. (1995); The Characterisation and Toxicity of Sediment Contaminated with Road Runoff; *Water Research*; **29(9):** 2043-2050.

Brenniman, G. R., Anver, M. R., Hartung, R. and Rosenburg, S. H. (1979); Effects of Outboard Motor Exhaust on Goldfish (*Carassius auretus*); *Journal of Environmental Pathology and Toxicology*; **2:** 1267-1281.

Broads Authority (1997); Broads Plan, 1997 - The Strategy and Management Plan for the Norfolk and Suffolk Broads; Broads Authority, 18 Colegate, Norwich, Norfolk, NR3 1BQ.

Broman, D., Naf, C., Lundbergh, I. and Zebuhr, Y (1990); An *In Situ* Study on the Distribution, Biotransformation and Flux of Polycyclic Aromatic Hydrocarbons (PAHs) in an Aquatic Food Chain (seston *Mytilus edulis* L. and *Somateria mollissima* L.) from the Baltic: An ecotoxicological perspective; *Environmental Toxicology and Chemistry*; **9(4):** 429-442.

Brown, D. W. and Adams, C. E. (1995); *Temporal Distribution of Craft Activity on Loch Lomond*; Draft copy of report to Scottish Sports Council, October 1995; University Field Station, Rowardennan, Glasgow, G63 0AW.

Burchan, W. E. and Jobes, M. (1995); *Nuclear and Particle Physics*; Longman Group Ltd.

Butcher, G. A. (1982); *The Effects of Outboard Engine Usage and Exhaust Emissions on the Aquatic Environment. A Review*; Province of British Columbia, Ministry of Environment, 1982.

Byrd, J. E. and Perona, M. J. (1980); The Temporal Variations of Lead Concentrations in a Freshwater Lake; *Water, Air and Soil Pollution*; **13:** 207-220.

Camper, N. D., Whitwell, T., Keese, R. J. and Riley, M. B. (1994); Herbicide Levels in Nursery Containment Pond Water and Sediments; *Journal of Environmental Horticulture*; **12(1):** 8-12.

Carls, M. G. and Rice, S. D. (1988); Sensitivity Differences Between Eggs and Larvae of Walleye Pollock (*Theragra chalcogramma*) to Hydrocarbons; *Marine Environmental Research*; **26(4):** 285-297.

Cavalieri, E. L., Higginbotham, S., Ramakrishna, N. V. S., Devanesan, P. D., Todorovic, R., Rogan, E. G. and Sqalmasi, S. (1991); Comparative Dose-Response Tumorigenicity Studies of Dibenzo (a) Pyrene versus 7, 12-Dimetnylbenz (a) Anthracene, Benzo (a) Pyrene and Two Dibenzo (a) Pyrene Dihydrodiols in Mouse Skin and Rat Mammary Gland; *Carcinogenesis (Eynsham)*; **12(10):** 1939-1944.

Chan, C.-C., Nien, C.-K., Tsai, C.-Y. and Her, G.-R. (1995); Comparison of Tail-Pipe Emissions from Motorcycles and Passenger Cars; *Journal of the Air and Waste Management Association*; **45:** 116-124.

Cheesham, R. V. and Wilson, A. L. (1989); A Manual on Analytical Quality Control for the Water Industry; revised by M. J. Gardner, June 1989.

Chmura, G. L. and Ross, N. W. (1978); *The Environmental Impact of Marinas and their Boats*; Department of Environmental Management Marine Advisory Service, Marine Memorandum, N. 45, University of Rhode Island, Narragansett.

Churms, S. C. (1992); Carbohydrates; In *Chromatography, 5th Edition*, pp. B229-B292; Ed. E. Heftmann; Elsevier, Amsterdam.

Clark, R. B. (1989); *Marine Pollution* (second edition); Clarendon Press, Oxford.

Clark, R. M., Vicory, A. H. and Goodrich, J. A. (1990); The Ohio River Oil Spill: A Case Study; *Journal of American Water Works Association*; **82(3):** 39-44.

Coates, S. W. and G. G. Lassanske (1990); *Measurement and Analysis of Gaseous Exhaust Emissions from Recreational and Small Commercial Marine Craft*; SAE paper; May 29, 1990.

Connell, D. W. and Miller, G. J. (1984); *Chemistry and Ecotoxicology of Pollution*; Wiley and Sons, Chichester.

Cooney, J. D. (1993); Freshwater Tests; In: *Fundamentals of Aquatic Toxicology*, pp. 71-102 (Chapter 2), Ed. G. M. Rand; Taylor and Francis Ltd., London.

CEC (Council of the European Community) (1994*a*); *Risk Assessment of Existing Substances*; Technical Guidance Documents in Support of the Commission Regulation (EEC) # 1488/94 on Risk Assessment for Existing Substances in Accordance with Council Regulation (EEC) # 793/93; Chapter 3 - Environmental Risk Assessment; European Commission, Brussels.

CEC (Council of the European Community) (1994*b*); *Scientific Advisory Committee to Examine the Toxicity and Ecotoxicity of Chemical Compounds* – Activity Report for 1992-1993; Report No. EUR 15674; European Commission, Brussels.

Cranwell, P. A. and Koul, V. K. (1989); Sedimentary Record of Polycyclic Aromatic and Aliphatic Hydrocarbons in the Windermere Catchment; *Water Research*; **23(3):** 275-283.

Curran, J. C. and Poodle, T. (1994); Aspects of the Hydrology and Hydrography of Loch Lomond; *Hydrobiologia*; **290:** 21-28.

Dahl, B. and Blanck, H. (1996); Toxic Effects of the Antifouling Agent, Irgarol-1051 on Periphyton Communities in Coastal Water Microcosms; *Marine Pollution Bulletin*; **32(4):** 342-350.

Dawodu, O, F. and Meisen, A. (1993); Gas Chromatographic Analysis of Alkanolamine Solutions Using Capillary and Packed Columns; *Journal of Chromatography*; **629(2):** 297-307.

Dean, J. H. and DeGraeve, G. M. (1986); A Screen Device to Eliminate "Floaters" in *Daphnia magna* Toxicity Tests; *Environmental Toxicology and Chemistry*; **5:** 1055-1057.

Doi, J. (1993); Complex Mixtures; In *Handbook of Ecotoxicology*, pp. 289-310; Ed, P. Calow; Blackwell Scientific Publications, London.

Dominy, P. J. and Williams, W. P. (1985); The Relationship Between Changes in the Redox State of Plastoquinone and Control of Excitation Energy Distribution in Photosynthesis; *FEBS Lett.*; **179(2):** 321-324.

Dorn, P. B. and Campernolle R. van (1993); Effluents; In: *Fundamentals of Aquatic Toxicology*, pp. 903-937 (Chapter 33), Ed. G. M. Rand; Taylor and Francis Ltd., London.

Dowson, P. H., Bubb, J. M. and Lester, J. N. (1993); Temporal Distribution of Organotins in the Aquatic Environment – 5 Years after the 1987 UK Retail Ban on TBT-Based Antifouling Paints; *Marine Pollution Bulletin*; **26(9):** 487-494.

Dowson, P. H., Bubb, J. M. and Lester, J. N. (1994); The Effectiveness of the 1987 Retail Ban on TBT-Based Antifouling Paints in Reducing Butyltin Concentrations in East Anglia, UK; *Chemosphere*; **28(5):** 905-910.

Dumbarton District Council, Stirling District Council, Central Regional Council, Strathclyde Regional Council and the Loch Lomond Park Authority (1996); *The Loch Lomond Local (Subject) Plan for Tourism, Recreation and Conservation*; Final Plan, February 1996.

Durand, J. P., Boscher, Y., Petroff, N. and Berthelin, M. (1987); Automatic Gas Chromatographic Determination of Gasoline Components: Application to Octane Number Determination; *Journal of Chromatography*; **395:** 229-240

East Loch Lomond Visitor Survey (1989); Final Report. Prepared by the Tourism Development Unit, December 1989.

East Midlands Regional Council for Sport and Recreation (1979); *Noise Test at the National Water Sports Centre, Holme Pierrepoint, Nottingham, 21 May 1979.*

Emans, H. J. B., Plassche, van der, E. J., Canton, J. H., Okkerman, P. C., and Sparenburg, P. M. (1993); Validation of Some Extrapolation Methods Used for Effect Assessment; *Environmental Toxicology and Chemistry*; **12:** 2139-2154.

English, J. N., Mc.Dermott, G. N. and Henderson, C. (1963); Pollutional Effects of Outboard Motor Exhaust - Laboratory Studies; *Journal of Water Pollution Control Federation*; **35(7):** 923-931.

Evans, K. M., Gill, R. A. and Robotham, P. W. J. (1990); The Source, Composition and Flux of Polycyclic Aromatic Hydrocarbons in Sediments of the River Derwent, Derbyshire, UK; *Water, Air and Soil Pollution*; **51(1-2):** 1-12.

Evershed, R. P. (1993); Combined Gas Chromatography-Mass Spectrometry; In *Gas Chromatography - a Practical Approach*, pp. 359-392; Ed. P. J. Baugh; IRL Press.

Fent, K. and Hunn, J. (1995); Organotins in Fresh-Water Harbors and Rivers – Temporal Distribution, Annual Trends and Fate; *Environmental Toxicology and Chemistry*; **14(7):** 1123-1132.

Ferrando, M. D. and Andreu, E. (1993); Feeding Behaviour as an Index of Copper Stress in *Daphnia magna* and *Brachionus calyciflorus*; *Comparative Biochemistry and Physiology C Comparative Pharmacology and Toxicology*; **106(2):** 327-331.

Finney, A. J. (1971); *Probit Analysis*, 3rd ed.; Cambridge University, Cambridge, UK.

Fowler, J. and Cohen, L. (1990); *Practical Statistics for Field Biology*; John Wiley and Sons, Chichester.

French, H. F. (1990); *Clearing the Air: A Global Agenda*; Worldwatch Paper #94, Library of Congress Catalog Number 89-51926.

Gala, W. R. and Giesy, J. P. (1992); Photo-induced toxicity of anthracene to the green alga, *Selenastrum capricornutum.; Archives of Environmental Contamination and Toxicology*; **23(3):** 316-323.

Garrad, P. N. and Hey, R. D. (1987); Boat Traffic, Sediment Resuspension and Turbidity in a Broadland River; *Journal of Hydrology*; **95:**289-297.

Gaur, J. P. and Singh, A. K. (1989); Comparative Studies on the Toxicity of Petroleum Oils and their Aqueous Extracts Towards *Anabaena doliolum*; *Proc. Indian Acad. Sci. (Plant Sci.)*; **99(5):** 459-466.

Gordon, D. C. Jr. and Keizer, P. D. (1974); *Estimation of Petroleum Hydrocarbons in Seawater by Fluorescence Spectroscopy: Improved Sampling and Analytical Methods*; Department of the Environment Fisheries and Marine Service, Marine Ecology Laboratories; Technical Report No. 481.

Goulden, C. E., Comotto, R. M., Hendrickson, J. A. Jr., Horning, L. L. and Johnson, K. L. (1982); *Proceedures and Recommendations for the Culture and Use of* Daphnia *in Bioassay Studies.*; Proceedings of the American Society of Testing and Materials Fifth Toxicology Symposium, Philadelphia, Pennsylvania USA; Special Technical Publication Number 7668; pp. 139-160.

Greentech Research (undated); *Positive Environmental Effects of Powerboats*; Report No. 900326; Greentech Research sprl, Voye du Four 23, B 6887, Herbeumont, Belgium.

Gschwend, P. M., Zafiriou, O. C., Mantoura, R. F. C., Schwarzenbach, R. P. and Gagosian, A. B. (1982); Volatile Organic Compounds at a Coastal Site. 1. Seasonal Variations; *Environ. Sci. Technol.*; **16:** 31-38.

Gudzinowicz, B. J., Gudzinowicz, M. J. and Martin, H. F. (1976); *Fundamentals of Integrated GC-MS*; Marcel Dekker Inc., New York.

Gunn, I. D. M., Bailey-Watts, A. E. and Lyle, A. A. (1994); Assessment of Eutrophication in Loch Lomond by Desk Analysis; *Hydrobiologia*; **290:** 51-52.

Habib, O. A. (1993); *Environmental and Seasonal Influences on Lake Phytoplankton Community Structure*; M.Sc. Thesis, Glasgow University, UK.

Habib, O. A., Tippett, R. and Murphy, K. J. (1997); Seasonal Changes in Phytoplankton Community Structure in Relation to Physico-Chemical Factors in Loch Lomond, Scotland; *Hydrobiologia*; **350:** 63-79.

Haddad, P. R. and Patsalides, E. (1992); Inorganic Species; In *Chromatography, 5th Edition*, pp. B1-B74; Ed. E. Heftmann; Elsevier, Amsterdam.

Hamer, M. (1994); Smarter Motors are Cleaner Motors; *New Scientist*, 3 December 1994, p. 24.

Hamilton, J. D. (1988); Recent Human Influences on the Ecology of Loch Lomond, Scotland; *Verh. Internat. Verein. Limnol.*; **23:** 403-413.

Hardy, J. T., Crecelius, E. A., Antrim, L. D., Kiesser, S. L., Broadhurst, V. L., Boehm, P. D., Steinhauer, W. G. and Coogan, T. H. (1990); Aquatic Surface Microlayer Contamination in Chesapeake Bay; *Marine Chemistry*; **28:** 333-351.

Harriman, G. E. (1993); The Role of Gas Chromatography in Petroleum Exploration; In *Gas Chromatography - a Practical Approach*, pp. 331-357; Ed. P. J. Baugh; IRL Press.

Harrison, R. M. and Peak, J. D. (1995); Global Disposition of Contaminants; In: *Handbook of Ecotoxicology*, pp. 633-651, Ed. D. J. Hoffman, B. A. Rattner, G. A. Burton, Jr. and J. Carins, Jr.; Lewis Publishers, London.

Haslam, S. M. (1990); *River Pollution: An Ecological Perspective*; John Wiley and sons, Chichester.

Hekmat, M., Latawiec, A. and Smith, R. (1994); Determination of Coal Tar Pitch Volatile Materials on Air Sampling Filters: Comparison of Gravimetric and Spectroscopic Techniques; *American Industrial Hygiene Association Journal*; **55(10):** 942-945.

Hellou, J., Upshall, C., Payne, J. F., Naidu, S. and Paranjape, M. A. (1993); Total Unsaturated Compounds and Polycyclic Aromatic Hydrocarbons in Molluscs Collected from Waters around Newfoundland; *Archives of Environmental Contamination and Toxicology*; **24(2):** 249-257.

Hellou, J., Payne, J. F., Upshall, C., Fancey, L. L. and Hamilton, C. (1994); Bioaccumulation of Aromatic Hydrocarbons from Sediments: A Dose-Response Study with Flounder *(Pseudopleuronectes americanus)*; *Archives of Environmental Contamination and Toxicology*; **27(4):** 477-485.

Horsfall, L., Jelinek, A. and Timms, B. V. (1988); The Influence of Recreation, Mainly Power Boating, on the Ecology of the Thirlmere Lakes, N.S.W., Australia; *Verh. Internat. Verein. Limnol.*; **23:** 580-587; Stuttgart, January 1988.

Hume, R. A. (1976); Reaction of Goldeneyes to Boating; *British Birds*; **69:** 178-179.

Huntley, S. L., Bonnevie, N. L. and Wenning, R. J. (1995); Polycyclic Aromatic Hydrocarbon and Petroleum Hydrocarbon Contamination in Sediment from the Newark Bay Estuary, New Jersey; *Archives of Environmental Contamination and Toxicology*; **28(1):** 93-107.

Hutchinson, G. E. (1957); *A Treatise on Limnology, Volume 1* (Geography, Physics and Chemistry); John Wiley and Sons, New York.

Ingersoll, C. G. (1993); Chapter 8 – Sediment Tests; In *Fundamentals of Aquatic Toxicology*, pp. 231-256; Ed. G. M. Rand; Taylor and Francis, London.

IWAAC (Inland Waterways Amenity and Advisory Council) (1983); *Waterway Ecology and the Design of Recreational Craft*; 122 Cleveland St., London.

Jackivicz, T. P. and Kuzminski, L. N. (1973) ; A Review of the Effects of Outboard Motor Exhaust on the Aquatic Environment; *Journal of Water Pollution Control Federation*; **45, 8:** 1759-1769.

Jemma, C. A., Shore, P. R. and Widdicombe, K. A. (1995); Analysis of C1 – C16 Hydrocarbons Using Dual-Column Capillary GC: Application to Exhaust Emissions from Passenger Cars and Motorcycle Engines; *Journal of Chromatographic Science*; **33:** 44-49.

Johansen, N. G., Ettre, L. S., and Miller, R. L. (1983); Quantitative Analysis of Hydrocarbons in Gasolines and Distillates: 1. Gas Chromatography; *Journal of Chromatography*; **256:** 393-417.

John, P. and Soutar, I. (1976); Identification of Crude Oils by Synchronous Scanning Excitation Spectrofluorimetry; *Analytical Chemistry*; **48(3):** 520-528.

John, P. and Soutar, I. (1981); Oil Spills: The Role of Luminescence Techniques; *Chemistry in Britain*; **17(6):** 278-286.

Johnson, B. T. and Romanenko, V. I. (1989); A Multiple Testing Approach for Hazard Evaluation of Complex Mixtures in the Aquatic Environment: The Use of Diesel Oil as a Model; *Environmental Pollution*; **58(2-3):** 221-236.

Johnstone, I. M., Coffey, B. T. and Howard-Williams, C. (1985); The Role of Recreational Boat Traffic in Interlake Dispersal of Macrophytes – a New Zealand Case Study; *Journal of Environmental Management*; **20(3):** 263-279.

Jones, M., Folt, C. and Guarda, S. (1991); Characterising Individual, Population and Community Effects of Sublethal Levels of Aquatic Toxicants: an Experimental Case Study Using *Daphnia*; *Freshwater Biology*; **26:** 35-44.

Juchelka, C. M. and Snell, T. W. (1995); Rapid Toxicity Assessment Using Ingestion Rate of Cladocerans and Ciliates; *Archives of Environmental Contamination and Toxicology*; **28:** 508-512.

Jüttner, F. (1994); Emission of Aromatic Hydrocarbons and Aldehydes into the Water by a Four-Stroke Outboard Motor: Quantitative Measurements; *Chemosphere*; **29(2):** 191-200.

Jüttner, F., Backhaus, D., Matthias, U., Essers, U., Greiner, R. and Mahr, B. (1995*a*); Emissions of Two- and Four-Stroke Outboard Engines. I. Quantification of Gases and VOC; *Water Research*; **29(8):** 1976-1982.

Jüttner, F., Backhaus, D., Matthias, U., Essers, U., Greiner, R. and Mahr, B. (1995*b*); Emissions of Two- and Four-Stroke Outboard Engines. II. Impact on Water Quality; *Water Research*; **29(8):** 1983-1987.

Keeton, W. T. and Gould, J. L. (1986); *Biological Science* (Fourth Edition); Norton, New York.

Kempinger, J. J., Otis, K. J. and Ball, J. R. (1998); Fish Kills in the Fox River, Wisconsin, Attributable to Carbon Monoxide from Marine Engines; *Transactions of the American Fisheries Society*; **127:** 669-672.

Khangarot, B. S., and Ray, P. K. (1989); Investigation of Correlation between Physicochemical Properties of metals and their toxicity to the Water Flea *Daphnia magna* Straus; *Ecotoxicology and Environmental Safety*; **18:** 109-120.

Kolpack, R. L., Mattson, J. S., Mark, H. B. Jr. and Yu, T.-C. (1971); Hydrocarbon Content of Santa Barbara Channel Sediments; In R. L. Kolpack (Ed.) *Biological and Oceanographical Survey of the Santa Barbara Channel Oil Spill, 1969-70, Volume II: Physical, Chemical and Geological Studies*; Allan Hancock Foundation, University of Southern California, Los Angeles.

Kooijman, S. A. L. M. (1981); Parametric Analyses of Mortality Rates in Bioassays; *Water Research*; **15:** 107-119.

Kuhn, R., Pattard, M., Pernak, K. and Winter, A. (1989*a*); Results of the Harmful Effects of Selected Water Pollutants (anilines, phenols, aliphatic compounds) to *Daphnia magna*; *Water Research*; **23(4):** 495-500.

Kuhn, R., Pattard, M., Pernak, K. and Winter, A. (1989*b*) ; Results of the Harmful Effects of Water Pollutants to *Daphnia magna* in the 21 day reproduction test; *Water Research*; **23(4):** 501-510.

Kuzminski, L. N., Ghan, H. B. S. and Roberts, J. L. (1972); *Studies on the Acute Toxicity of Two-Cycle Outboard Motor Exhausts to Selected Fish Species.*; Progress Report for Division of Water Pollution Control, Massachusetts Water Resources Commission.; Report No. EVE-28-72-1; September 1972.

Lagler, K. F., Hazzard, A. S., Hazen, W. E. and Tomkins, W. A. (1950); Outboard Motors in Relation to Fish Behaviour, Fish Production and Angling Success. *Transactions of the Fifteenth North American Wildlife Conference*; **15:** 280-303.

LDNPA (Lake District National Park Authority) (1994); *Boat Use Survey on Lake Windermere, 1993*; LDNPA, Murley Moss, Oxenholme Road, Kendal, Cumbria, LA9 7RL, UK.

Lakowicz, J. R. (1983); *Principles of Fluorescence Spectroscopy*; Plenum Press, London.

Larsson, P. G., Harboe, A. and Aalen, O. O. (1981); A Computer Programme for the Evaluation of Dose-Response Data; *Norwegian Institute for Public Health Annals*, **Vol. 4, #1**, June 1981; ISSN 0332-5652.

Law, R. J., Fileman, T. W. and Portmann, J. E. (1988); *Methods for the Analysis of Hydrocarbons in Marine and Other Samples*; Aquatic Environmental Protection: Analytical Methods # 2; Ministry of Agriculture, Fisheries and Food (MAFF), Directorate of Fisheries Research.

Leithe, W. (1973); *Analysis of Organic Water Pollutants*; Ann Arbor Science Publishers, Inc., PO Box 1425, Ann Arbor, Michigan, 48106, USA.

Lewis, M. A. (1992); The Effect of Mixtures and Other Environmental Modifying Factors on the Toxicities of Surfactants to Freshwater and Marine Life; *Water Research*; **26(8):** 1013-1023.

Liddle, M. J., and Scorgie, H. R. A. (1980); The Effects of Recreation on Freshwater Plants and Animals: a Review; *Biological Conservation*; **17:** 183-206.

Loch Lomond and the Trossachs Working Party [chariman: Sir Peter Hutchison] (1993); *The Management of Loch Lomond and the Trossachs*; the report of the Loch Lomond and the Trossachs Working Party to the Secretary of State for Scotland, July, 1993.

López de Alda-Villaizan, M. J., Garcia, F. S., Lage-Yusty, M. A. and Lozano, J. S. (1995); Synchronous Spectrofluorimetric Determination of Total Amounts of the Six Polycyclic Aromatic Hydrocarbons Officially Designated as Indicators of Drinking Water Quality; *Journal of AOAC International*; **78(2):** 402-406.

Loudoun, G. M. (1988); *Organic Chemistry* (Second Edition); The Benjamin/Cummings Publishing Company Ltd., Wokingham.

McDonald, S. J., Kennicutt, M. C. II and Brooks, J. M. (1992); Evidence of Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Fish from the Antarctic Peninsula; *Marine Pollution Bulletin*; **25:** 313-317.

McKee, R. H., and Plutnick, R. T. (1989); Carcinogenic Potential of Gasoline and Diesel Engine Oils; *Fundamental and Applied Toxicology*; **13(3):** 545-553.

McLafferty, F. W. (1980); *Interpretation of Mass Spectra (Third Edition)*; University Science Books, 20 Edgehill Road, Mill Valley, CA 94141, USA.

Maher, W., Tomlins, C. and Furlonger, J. (1990); Petroleum Hydrocarbon Pollution of Lake Burley Griffin; *Oil and Chemical Pollution*; **6:** 81-90.

Maltby, L., Naylor, C. and Calow, P. (1990); Effect of Stress on a Freshwater Benthic Detritivore: Scope for Growth in Gammarus pulex; *Ecotoxicology and Environmental Safety*; **19:** 285-291.

Mantoura, R. F. C., Gshwend, P. M., Zafiriou, O. C. and Clarke, K. R. (1982); Volatile Organic Compounds at a Coastal Site: 2. Short Term Variations.; *Environ. Sci. Technol.*; **16(1):** 38-45.

Marmontel, M., Humphrey, S. R. and O'Shea, T. J. (1997); Population Viability Analysis of the Florida Manatee (*Trichechus manatus latirostris*), 1976-1991; *Conservation Biology*; **11(2):** 467-481.

Marsalek, J. and Schroeter, H. (1988); Annual Loadings of Toxic Contaminants in Urban Runoff from the Canadian Great Lakes Basin; *Water Pollution Research Journal of Canada*; **23(3):** 360-378.

Mastran, T. A., Dietrich, A. M., Gallagher, D. L. and Grizzard, T. J. (1994); Distribution of Polyaromatic Hydrocarbons in the Water Column and Sediments of a Drinking Water Reservoir with respect to Boating Activity; *Water Research*; **28(11):** 2353-2366.

Mele A. (1993).; *Polluting for Pleasure*; Norton, New York.

Merck Index (1996); An Encyclopedia of Chemicals, Drugs and Biologicals; Merck Research Laboratories, Division of Merck and Co. Ltd., Whitehouse Station, N.J., U.S.A.

MEWAM (Methods for the Examination of Waters and Associated Materials) (1988); *Analysis of Hydrocarbons in Waters - A Review and an Ultra-Violet Fluorescence Spectrometric Method, 1988*; Method No. 128; HMSO, London.

Meteorological Office (Met. Office) data (1998); Met. Office, Bracknell, Berkshire.

Miller, G. C. and Fiore, M. (1997); *Preliminary Study on Gasoline Constituents in Lake Tahoe, Summer, 1997*; Submitted to the Lahonter Regional Water Quality Control Board, Environmental and Resource Sciences, University of Nevada, Reno, 89557.

Mokry, L. E. and Hoagland, K. D. (1990); Acute Toxicities of Five Synthetic Pyrethroid Insecticides to *Daphnia magna* and *Ceriodaphnia dubia*; *Environmental Toxicology and Chemistry*; **9(8):** 1045-1052.

Montz, W. E. Jr., Puyear, R. L. and Brammer, J. D. (1982); Identification and Quantification of Water Soluble Hydrocarbons by Two-Cycle Outboard Motors; *Archives of Environmental Contamination and Toxicology*; **11:** 561-565.

Muir, G. D. (1977); *Hazards in the Chemical Laboratory (2nd. Edition)*; The Chemical Society, London.

Mukherjee, B. and Pankajakshi, G. V. N. (1995); The Impact of Detergents on Plankton Diversity in Freshwaters; *Journal of Environmental Biology*; **16(3):** 211-218.

Muratori, A. Jr. (1968); How Outboards Contribute to Water Pollution; *The Conservationist*; **22:** 6-8.

Murphy, K. J., Eaton, J. W. and Hyde, T. M. (1982); The Management of Aquatic Plants in a Navigable Canal System Used for Amenity and Recreation; *Proceedings of the E.W.R.S. 6th. Symposium on Aquatic Weeds*, Wageningen, The Netherlands.

Murphy, K. J. and Eaton, J. W. (1983); Effects of Pleasure Boat Traffic on Macrophyte Growth in Canals.; *Journal of Applied Ecology*; **20:** 713-729.

Murphy, K. J., Willby, N. J. and Eaton, J. W. (1993); Ecological Impacts and Management of Boat Traffic on Navigable Inland Waterways; In *The Ecological Basis for River Management*; ed. Harper, D. M. and Ferguson, A. J. D.; John Wiley and Sons.

Murphy, K. J., Hudson, K. D. and Mitchell, J. (1994); Freshwater and Wetland Plant Communities of Loch Lomond; *Hydrobiologia*; **290:** 63-74.

Nanson, G. C., Vonkrusentierna, A., Bryant, E. A. and Renilson, M. R. (1994); Experimental Measurements of River-Bank Erosion Caused by Boat-Generated Waves on the Gordon River, Tasmania; *Regulated Rivers - Research and Management*; **9(1):** 1-14.

NRC (National Research Council), 1985; *Oil in the Sea: Inputs, Fates and Effects*; National Academy Press, Washington DC.

O'Shea, T. J., Beck, C. A., Bonde, R. K., Kochman, H. I. and Odell, D. K. (1985); An Analysis of Manatee (*Trichechus manatus*) Mortality Patterns in Florida, USA, 1976-1981; *J. Wildl. Manage.*; **49(1):** 1-11.

Ohio River Valley Water Sanitation Committee (1970); *Pollution Control Standard No. 1-70*; 414 Walnut Street, Cincinnati, Ohio, 45202.

Olson, K. L., Sinkevitch, R. M. and Sloane, T. M. (1992) ; Speciation and Quantitation of Hydrocarbons in Gasoline Engine Exhaust; *Journal of Chromatographic Science*; **30:** 500-508.

Oost, R. van der, Schooten, F. J. van, Ariese, F., Heida, H., Satumalay, K. and Vermeulen, N. P. E. (1994); Bioaccumulation, Biotransformation and DNA Binding of PAHs in Feral Eel (*Anguuilla anguilla*) Exposed to Polluted Sediments: A Field Survey; *Environmental Toxicoology and Chemistry*; **13(6):** 859-870.

Organisation for Economic Cooperation and Development (OECD) (1984);Daphnia *sp., Acute Immobilisation Test and Reproduciton Test – guideline adopted 4 April 1984*; OECD Guidelines for Testing of Chemicals (1981-), #202, pp. 1-5; OECD, 2 Rue André Pascal, 75773, Cedex 66, Paris, France.

Organisation for Economic Cooperation and Development (OECD) (1992); *Report of the OECD Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment*; Environmental Monograph #59, 43pp.; OECD, 2 Rue André Pascal, 75773, Cedex 66, Paris, France.

Page, D. S., Foster, J. C., Fickett, P. M. and Gilfillan, E. S. (1988); Identification of Petroleum Sources in an Area Impacted by the *Amoco Cadiz* Oil Spill; *Marine Pollution Bulletin*; **19**: 107-115.

Payne, S. J. and Hey, R. D. (1982); *River Management to Reduce Bank Erosion*, Yare and Bure River System; BARS 4; Broads Authority, Norfolk and Suffolk, Thomas Harvey House, 18 Colegate, Norwich, Norfolk, NR3 1BQ.

Pearce, F. (1996); A Heavy Responsibility; *New Scientist*, 27 July 1996, p. 12.

Persoone, G. and Janssen, C. R. (1993); Freshwater Invertebrate Toxicity Tests; In *Handbook of Ecotoxicology*, pp. 378-396; Ed, P. Calow; Blackwell Scientific Publications, London.

Peterson, D. R. (1994); Calculating the Aquatic Toxicity of Hydrocarbon Mixtures; *Chemosphere*; **29(12):** 2493-2506.

Pham, T., Lum, K. and Lemieux, C. (1993); Sources of PAHs in the St. Lawrence River (Canada) and their Relative Importance; *Chemosphere*; **27(7):** 1137-1149.

Phillips, D. J. H. (1993); Bioaccumulation; In *Handbook of Ecotoxicology*, pp. 378-396; Ed, P. Calow; Blackwell Scientific Publications, London.

Pirie, D. J.; Organic Chemist, Scottish Environmental Protection Agency (SEPA), West Region, Rivers House, The Murray, East Kilbride, G75 0LA; personal communication to the author on August 21st. 1996.

Poole, C. F. and Poole, S. K. (1992); Gas Chromatography; In *Chromatography, 5th Edition*, pp. A393-A447; Ed. E. Heftmann; Elsevier, Amsterdam.

Pople, A., Simpson, R. D and Cairns, S. C. (1990); An Incident of Southern Ocean Oil Pollution: Effects of a Spillage of Diesel Fuel on the Rocky Shore of Macquarie Island (Sub-Antarctic); *Aust. J. Mar. Freshwater. Res.*; **41:** 603-620.

Porte, C and Albaieges, J. (1994); Bioaccumulation Patterns of Hydrocarbons and Polychlorinated Biphenyls in Bivalves, Crustaceans and Fish; *Archives of Environmental Contamination and Toxicology*; **26(3):** 273-281.

Poulton B. C., Finger, S. E. and Humphrey, S. A. (1997); Effects of a Crude Oil Spill on the Benthic Invertebrate Community in the Gasconade River, Missouri; *Archives of Environmental Contamination and Toxicology*; **33:** 268-276.

Rand, G. M.(1993); *Fundamentals of Aquatic Toxicology*, Ed. G. M. Rand; Taylor and Francis Ltd., London.

Rand, G. M., Wells, P. G. and McCarty, L. S. (1993); Introduction to Aquatic Toxicology; In: *Fundamentals of Aquatic Toxicology*, pp. 3-70 (Chapter 1), Ed. G. M. Rand; Taylor and Francis Ltd., London.

Rattner, B. A. and Heath, A. G. (1995); Environmental Factors Affecting Contaminant Toxicity in Aquatic and Terrestrial Vertebrates; In: *Handbook of Ecotoxicology*, pp. 519-535, Ed. D. J. Hoffman, B. A. Rattner, G. A. Burton, Jr. and J. Carins, Jr.; Lewis Publishers, London.

Rees, J. and Tivy, J. (1978); Recreational Impact on Scottish Lochshore Wetlands; *Journal of Biogeography*; **5**: 93-108.

Rice, C. D. and Weeks, B. A. (1990); The Influence of in vitro Exposure to Tributyltin on Reactive Oxygen Formation in Oyster Toadfish Macrophages; *Archives of Environmental Contamination and Toxicology*; **19:** 854-857.

Ritesma, R. (1994); Dissolved Butyltins in Marine Waters of the Netherlands 3 Years after the Ban; *Applied Organometallic Chemistry*; **8(1):** 5-10.

Rousch, J. M. and Sommerfield, M. R. (1998); Liquid-Gas Partitioning of the Gasoline Oxygenate Methyl Tert-Butyl Ether (MTBE) Under Laboratory Conditions and its Effect on Growth of Selected Algae; *Archives of Environmental Contamination and Toxicology*; **34(1):** 6-11.

Rufli, H., Fisk, P. R., Girling, A. E., King, J. M. H., Länge, R., Lejeune, X., Stelter, N., Stevens, P., Suteau, J., Tapp., J., Thus, J., Versteeg, D. J. and Niessen, H. J. (1998); Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances and Interpretation and Use of Data; *Ecotoxicology and Environmental Safety*; **39(2):** 72-77.

Sanders, G., Jones, K. C., Hamilton-Taylor, J. and Doerr, H. (1993); Concentrations and deposition fluxes of polynuclear aromatic hydrocarbons and heavy metals in the dated sediments of a rural English lake; *Environmental Toxicology and Chemistry*; **12(9):** 1567-1581.

Sauer, T. C., Brown, J. S., Boehm, P. D., Aurand, D. V., Michel, J. and Hayes, M. O. (1993); Hydrocarbon Source Identification and Weathering Characteristics of Intertidal and Subtidal Sediments Along the Saudi Arabian Coast after the Gulf War Oil Spill; *Marine Pollution Bulletin*; **27**:117-134.

Schwarzenbach, R. P., Bromund, R. H., Gschwend, P. M. and Zafiriou, O. C. (1978); Volatile Organic Compounds in Coastal Seawater; *Organic Geochemistry*; **1:** 93-107.

Schwarzenbach, R. P., Gschwend, P. M. and Imboden, D. M. (1993); *Environmental Organic Chemistry*; Wiley, New York.

Shackley, M. (1992); Manatees and Tourism in Southern Florida: Opportunity or Threat?; *J. Environ. Manage.*; **34(4):** 257-265.

Shchekaturina, T. L., Khesina, A. L., Mironov, O. G. and Krivosheeva, L. G. (1995); Carcinogenic Polycyclic Aromatic Hydrocarbons in Mussels from the Black Sea; *Marine Pollution Bulletin*; **30(1):** 38-40.

Sherma, J. (1992); Pesticides; In *Chromatography, 5th Edition*, pp. B513-B554; Ed. E. Heftmann; Elsevier, Amsterdam.

Shiu, W. Y., Maijanen, A., Ng, A. L. Y. and Mackay, D. (1988); Preparation of Aqueous Solutions of Sparingly Soluble Organic Substances. II: Multicomponent Systems – Hydrocarbon Mixtures and Petroleum Products; *Environmental Toxicology and Chemistry*; **7:** 125-137.

Sigma-Aldrich Chemical Safety Data (1995); Sigma Chemical, Fancy Road, Poole, Dorset.

Siron, R., Giusti, G., Berland, B., Morales-Loo, R. and Pelletier, E. (1991); Water-Soluble Petroleum Compounds: Chemical Aspects and Effects on the Growth of Microalgae; *The Science of the Total Environment*; **104 (1991):** 211-227.

Skoog, D. A., Holler, F. J. and Nieman, T. A. (1998); *Principles of Instrumental Analysis*; Saunders College Publishing, London.

Snyder, L. R. (1968); *Principles of Adsorption Chromatography*; Edward Arnold.

Soares, A. M. V. M., Baird, D. J. and Calow, P. (1992); Interclonal Variation in the Performance of *Daphnia magna* Straus in Chronic Bioassays.; *Environmental Toxicology and Chemistry*; **11(10):** 1477-1483.

Southworth, G. R., Beauchamp, J. J. and Schmeider, P. K. (1978); Bioaccumulation Potential of Polycyclic Aromatic Hydrocarbons in *Daphnia pulex*; *Water Research*; **12:** 973-977.

Spacie, A., McCarty, L. S. and Rand, G. M. (1993); Chapter 5 - Bioaccumulation and Bioavailability in Multiphase Systems; In *Fundamentals of Aquatic Toxicology*, pp. 493-521; Ed. G. M. Rand; Taylor and Francis, London.

Steidl, R. J. and Anthony, R. G. (1996); Responses of Bald Eagles to Human Activity During the Summer in Interior Alaska; *Ecological Applications*; **6(2):** 482-491.

Stephen, H, and Stephen, T. (1963); *Solubilities of Inorganic and Organic Compounds, Vol. 1* (Binary Systems, Part 1); Pergamon Press, Oxford.

Stephenson, G. L., Kaushik N. K. and Solomon, K. R. (1991); Acute Toxicity of Pure Pentachlorophenol and a Technical Formulation to Three Species of *Daphnia*; *Archives of Environmental Contamination and Toxicology*; **20(1):** 73-80.

Stolpe, N. (1992); *A Survey of Potential Impacts of Boating Activity on Estuarine Productivity*; presented at: Marine Engines and Vessels Public Workshop, Ann Arbor, Michigan.

Sutherland, A. J. and Ogle, D. G. (1975); Effects of Jet Boats on Salmon Eggs; *New Zealand Journal of Marine and Freshwater Research*; **9(3):** 273-282.

Takada, H., Onda, T. and Ogura, N. (1990); Determination of Polycyclic Aromatic Hydrocarbons in Urban Street Dusts and Their Source Materials by Capillary Gas Chromatography.; *Environmental Science and Technology*; **24(8):** 1179-1186.

Taylor, G., Baird, D. J. and Soares, A. M. V. M. (1998); Surface Binding of Contaminants by Algae: Consequences for Lethal Toxicity and Feeding to *Daphnia magna* Straus; *Environmental Toxicology and Chemistry*; **17(3):** 412-419.

Tetlow, J. A. (undated); *Detergents: Their Impact on the Environment*; Anglian Water Authority, Norwich.

Tippett, R. (1994); An Introduction to Loch Lomond; *Hydrobiologia*; **290:** xi-xv.

Tuite, C. H., Owen, M. and Paynter, D. (1983); Interaction between Wildfowl and Recreation at Llangorse Lake and Talybont Reservoir, South Wales.; *Wildfowl*; **34:** 48-63.

Tunstall, S., Fordham, M., Green, C. and House, M. (1997); Public Perception of Freshwater Quality with Particular Reference to Rivers in England and Wales; In: *Freshwater Quality: Defining the Indefinable*, pp. 39-58, Ed. P. J. Boon and D. J. Howell; The Stationery Office, Edinburgh.

Turrell, W. R. (1994); Modelling the *Braer* Oil Spill: A Retrospective View; *Marine Pollution Bulletin*; **28(4):** 211-218.

UK CEED (UK Centre for Economic and Environmental Development) (1993); *Waterskiing and the Environment - A Literature Review*; UK CEED, 3e King's Parade, Cambridge, CB2 1SJ.

US EPA (United States Environmental Protection Agency) (1991); *Nonroad Engine and Vehicle Study*, Report PB92-126960, Washington D.C. : National Technical Information Service, US Department of Commerce.

Varney, H. and Crooks, J. A. (1989); *The Effects of Recreation on Wildfowl at Llangorse Lake, August - September 1989*; Brecon Beacons National Park Authority, 7 Glamorgan St., Brecon, Powys, LD3 7DP.

Wachs, B., Wagner, H. and van Donkelaar, P. (1992); Two-Stroke Engine Lubricant Emissions in a Body of Water Subjected to Intensive Outboard Motor Operation; *The Science of the Total Environment*; **116:** 59-81.

Waddell, Y. T. (1993); *Feeding Responses of* Daphnia magna *Under Exposure to Toxic Chemicals*; Ph.D. thesis, University of Sheffield, UK.

Wade, T. L., Velinsky, D. J., Reinharz, E. and Schlekat, C. E. (1994); Tidal River Sediments in the Washington, D.C. Area: II. Distribution and Sources of Organic Contaminants; *Estuaries*; **17(2):** 321-333.

Walker, S. E. (1994); Tourism and Recreation. pp. 333-343; In: *The Fresh Waters of Scotland* (P. S. Maitland, P. J. Boon and D. J. Mc. Lusky eds.); Wiley, Chitchester.

Wang, Z., Fingas, M. and Li, K. (1994); Fractionation of a Light Crude Oil and Identification and Quantitation of Aliphatic, Aromatic and Biomarker Compounds by GC-FID and GC-MS, Part II; *Journal of Chromatographic Science*; **32:** 367-373.

Wardlaw, A. C. (1985); *Practical statistics for Experimental Biologists* (Chapter 5); John Wiley and sons.

Weast, R. C. (1988); *CRC Handbook of Chemistry and Physics, 69th edition (1988-1989)*; CRC Press Inc., Boca Raton, Florida, USA.

Windermere Steering Committee [chairman: Mrs. C. Thornton](1981); *Windermere: a Management Plan for the Lake – Final Recommendations*; South Lakeland District Council, Stricklandgate House, Stricklandgate, Kendal, LA9 4QQ.

Wishkovsky, A., Matthews, E. S. and Weeks, B. A. (1989); Effect of Tributyltin on the Chemiluminescent Response of Phagocytes from Three Species of Estuarine Fish; *Archives of Environmental Contamination and Toxicology*; **18:** 826-831.

Wright, D. A. and Zamuda, C. D. (1991); Copper Contamination in the Patuxent River, Maryland; *Hydrobiologia*; **215(1):** 31-41.

Yamauchi, A., Matsumoto, N., Nakagawa, H., Ohotuka, T., Yamazaki, H. and Kakiuchi, Y. (1989); Determination of Polycyclic Aromatic Hydrocarbons in River Waters by Blue-Cotton Adsorption Method; *Eisei Kagaku*; **35(4):** 283-290.

Appendix I: Hydrocarbon Analysis Techniques

A.1: Introduction

A number of different techniques have been used in previous studies, to analyse water for hydrocarbons (HCs). Although it was possible to gain at least limited access to all of these analytical methods, an optimum method was needed for the current study. Thus, it was necessary to review the alternative methods of HC analysis, which is carried out here along with the reasons for choosing the most appropriate methods for the current study.

A.2: Review of Current Methods for HC Analysis

A.2.1: Gravimetric Analysis

The simplest method for the analysis of hydrocarbons in environmental samples is gravimetric analysis which has been used in previous studies, for example to measure hydrocarbons (HCs) in coal tar pitch vapour (Hekmat *et al.*, 1994) and weathered crude oil in sediment (Sauer *et al.*, 1993). Gravimetric analysis involves the extraction of hydrocarbons using an appropriate volatile solvent (for example hexane, C_6H_{14} or dichloromethane CH_2Cl_2) and the complete evaporation of the solvent and subsequent determination of the weight of the remaining hydrocarbon material. Unless, as in the aforementioned studies, relatively non-volatile substances are being measured, this method is not very sentitive, as many volatile compounds would themselves evaporate, along with the solvent. Also, the method is not very specific to hydrocarbons, as some non-hydrocarbon extractable material may be measured as well. In addition, particular compounds in the samples cannot be identified.

A.2.2: Infra-red Spectroscopic Analysis

A more precise method to analyse petroleum hydrocarbons is infra red (IR) spectroscopy (as used in Wachs *et al.*, 1992). This method is based on the absorbance of energy from electro-magnetic (EM) radiation at wavelengths within the IR range of the spectrum (approximately 1-5 μm), either by Carbon-Hydrogen or Carbon-Carbon bonds. Different wavelength bands cause absorbance by molecules with different bond types. Prior to analysis, the sample is extracted using tetrachloromethane (CCl_4 or Carbon tetrachloride) and the total amount of hydrocarbons is determined using appropriate standards. This method is more specific to petroleum hydrocarbons than gravimetric analysis. Although some indication of the relative proportions of different hydrocarbon compounds can be gained by analysing the different wavelength bands (corresponding to the different bond types and hence different hydrocarbon compounds), particular hydrocarbon compounds are not identified. IR spectroscopy is a standard procedure for quantification of HCs in environmental samples (ASTM, 1980*a*) and has been utilised in previous studies on oil pollution (Kolpack *et al.*, 1971) and toxicity of oil to marine crustaceans (Anderson *et al.*, 1980).

A.2.3:Fluorescence Spectroscopy

Fluorescence spectroscopy is a technique in widespread use these days for hydrocarbon analysis and it is based on the fact that all aromatic hydrocarbons fluoresce when targeted with ultra-violet (UV) or visible EM radiation of appropriate wavelengths (200-500 nm). Prior to analysis, samples are extracted using a suitable solvent - usually cyclohexane (C_6H_{12}), pentane (C_5H_{12}) or dichloromethane (CH_2Cl_2 or DCM). The sample is then subjected to EM radiation of specific wavelengths and the fluorescence emitted by the aromatic HCs (the quantum yield) is then measured at the requisite (always longer) emission wavelength, with an appropriate fluorimeter. Hydrocarbon concentrations are measured using appropriate standards, of the same or similar chemical composition to the samples measured. Different excitation and emission wavelengths are used for different classes of aromatic hydrocarbons.

The technique has been refined in Synchronous Scanning Fluorescence Spectroscopy (SSFS), whereby the sample is targeted by a range of scanned excitation wavelengths, with the fluorescence emission measured at a constant difference to (or offset from) the excitation wavelength. This technique can give a characteristic fluorescence "fingerprint" for any oil, and in its most refined form, it has great resolving power for distinguishing different oils and has been used successfully to identify oil in marine pollution incidents (John and Soutar, 1976, 1981). However, although this technique can give precise information on the types of oil analysed and provides some information on the types of hydrocarbons present, no information on particular compounds is given and none at all about the aliphatic compounds (which do not significantly fluoresce). A detailed account of Fluorescence Spectroscopy is given in Lakowicz (1983).

A.2.4: Gas Chromatography (GC)

A.2.4.1: General

One method in widespread use for HC analysis is Gas Chromatography (GC). GC is a sophisticated separation process and can be used in the analysis of a variety of fluid mixtures. It is of vital importance in pollution analysis, particularly useful in the analysis of complex mixtures which are often found in nature. The actual process of GC involves the introduction of samples into a heated column, lined with an appropriate adsorbant material or stationary phase and with a flowing inert carrier gas, or moving phase. Compounds are separated by differential partitioning between the stationary and moving phases, with temperature programming. Different films are used for substances of corresponding different polarity (Poole and Poole, 1992).

Either a capillary column or a packed column may be used. A capillary column is a very long (typically 30 m or more) narrow (usually with an internal diameter (i.d.) of less than 0.5 mm) tube internally coated with a thin film (the stationary phase). A packed column is a shorter (2 m or so) wider (i.d. greater than 2 mm) column packed

with silica beads (of approximate diameter 100-300 μm) coated with the stationary phase. Both types of column achieve the same effect: compounds are separated by differential partitioning between the stationary and moving phases. Compounds of low molecular weight and boiling point and those of opposing polarity to the stationary phase (i.e. those which adhere least to it), generally pass through the column most quickly and are detected before those of higher molecular weight and boiling point and those with similar polarity to the stationary phase. The time taken for a particular compound to pass through the column is the retention time (RT). However, the order of elution (i.e. the sequence in which the compounds emerge from the column) can vary according to the separation conditions.

Previous work (Gudzinowicz *et al.*, 1976; Poole and Poole, 1992; Dawodu and Meisen, 1993) has shown that capillary columns are more sensitive and have greater resolving power than packed columns, particularly for the analysis of complex mixtures (Best and Dawson, 1993).

A.2.4.2: GC Injection Methods

There are several established methods for the introduction of a sample to the column in GC analysis, with three of the most usual being:

1) "on-column" injection.
2) "split" injection.
3) "splitless" injection.

In "split" injection, the sample is vapourised and only a very small proportion is actually passed into the column with the rest expelled through a valve. "Splitless" injection involves the use of a larger proportion than that used in split injection. Since this larger proportion is quantifiable, this method benefits from greater reproducibility and precision. The former method is ideal for analysis of samples with compounds present in high concentrations, such as pure petrol, (e.g. Johansen *et al.*, 1983) while the latter is most appropriate for samples in which particular chemical compounds are found

in intermediate concentration, for example in some types of crude oil analyses (Harriman, 1993).

"On-column injection involves injecting the entire sample into the capillary column. This method has a number of advantages:

- Since the whole sample is used, the analysis is very comprehensive, with no non-volatile compounds lost in the injection process.
- Sensitivity is high, and this method is most appropriate for very dilute samples which typically occur in environmental analysis.

A.2.4.3: Detection and Identification of Hydrocarbons

A range of different detection apparatus can be used for the identification and quantification of specific chemicals, separated by GC, with one of the most often used being the Flame Ionisation Detector (FID; see Skoog et al., 1998 for detailed descriptions of FIDs and other detectors widely used nowadays). This is most useful in the accurate quantitative analysis of highly concentrated (sometimes completely undiluted) samples. For example, in a study by Johansen *et al.* (1983) the constituents of several different types of commercially sold petrols were quantified. Other work involving the use of GC-FID includes analyses of crude oil (e.g. Page *et al.* 1988), oil spills (Sauer *et al.* 1993) and gaseous emissions from automobiles (Olson *et al.* 1992). In all of these studies capillary columns were used, and compounds were identified by comparing their retention times with those of known standards.

FIDs have also been used in two previous analyses of powerboat pollution: Montz *et al.* (1982) used packed column GC with FID in the analysis of outboard motor exhaust-polluted water (EPW). In that study, eight hydrocarbon compounds were identified and quantified with three others tentatively identified. The Boating Industry Associations (1975) also used an FID in the analysis of total gaseous powerboat exhaust HCs, but the identities of only four compounds were given.

An alternative detection device is a mass spectrometer, the the basic components of which are shown in Fig. A.1. Mass spectrometry (MS) involves the fragmentation and ionisation of each compound and the sorting of these ionic fragments by a mass analyser. The resultant fragment patterns are then amplified and recorded, using a suitable recording device. Compounds may be ionised using a variety of methods with three of the most common being:

(1) electron impact ionisation

(2) chemical ionisation, using positively charged reagent gases

(3) electrical field ionisation

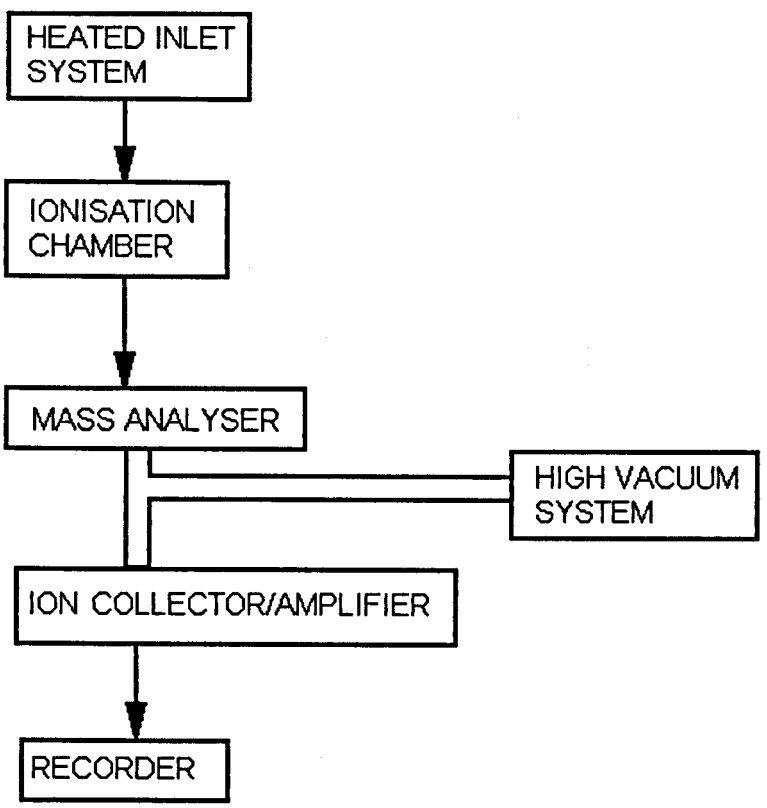

Fig. A.1: *Schematic diagram, showing the basic components of a mass spectrometer (from Gudzinowicz et al., 1976).*

There are also a number of different types of mass analyser with the two most commonly used being quadrupole and magnetic plate mass analysers. In a quadrupole mass analyser (which was the type used in this study), there are two stages. Firstly, a pre-filter removes most of the non-target ionic fragments. This pre-filter consists of four short metal rods with radio frequency voltages, which deflect most of the unwanted

ionic fragments and prevent them from reaching the second stage. This second stage is the mass analyser proper and consists of four longer rods with radio frequency voltage and also DC (direct current) voltage. This combination of voltages allows the passage of only one type of ionic fragment through the mass analyser at any particular instant. The voltages can be held constant to allow ions of only one mass or a few specific masses through (single ion recording or selective ion recording respectively) or scanned to allow ions of a wide range of masses through, separated in time.

In magnetic plate mass spectrometry (MS), the fragments are accelerated between a series of magnetic plates with varying magnetic field, which also selectively restricts the passage of ions. In both methods, the ion fragments are separated according to their mass/charge (m/z) ratio. In most ion fragments, $z = 1$ and the m/z ratio is simply the mass of each fragment in atomic mass units (a.m.u.).

The fragments are then detected by means of an electron multiplier. The ions strike a conversion dynode (which is a charged metal plate) and produce electrons on impact. These electrons then strike an aluminised phosphor plate, which in turn emanates light. These light signals are then electronically enhanced and ionic fragment patterns constructed. Every compound has its own distinctive fragment pattern or mass spectrum, and these mass spectra can be used to accurately identify any compound, using standards and computer stored library spectra, often containing spectra for tens of thousands of different compounds. Three sample mass spectra of organic compounds are shown in Appendix II and the salient features of these mass spectra are discussed. A description of modern GC-MS is given in Evershed (1993).

A.3: Conclusion: Methods Actually Chosen

In the current study, there were two main objectives which required precise analysis of HCs:

1) the detailed chemical characterisation of outboard motor exhaust HCs (Ch. 3).
2) the completion of a large-scale water quality survey of Loch Lomond involving the analysis of powerboat exhaust HCs (Ch. 5).

For the former objective, it was necessary to gain as much information as possible from samples of water, artificially contaminated by outboard motor exhaust and also to analyse a limited number water samples taken from Loch Lomond. For this analysis, capillary column GC-MS, with on-column injection (see Ch. 3, Table 3.1) was chosen as it was highly sensitive, comprehensive (on-column injection does not discriminate against non-volatiles) and gave information on particular compounds present in the samples. Since only a few samples were analysed, the lengthy time periods necessary for analysis were immaterial.

For the large-scale sampling programme, a fast method was necessary, ruling out any GC methods. Gravimetric analysis was also ignored, due to its lack of sensitivity and discrimination against volatile compounds. IR spectroscopy was considered, as it is specific to different classes of HCs, including volatile aromatics and is reasonably quick. However, the sensitivity was inadequate to detect the expected low levels of HCs occurring in environmental samples. The chosen method was fluorescence spectroscopy, which combines the sensitivity of GC-MS with the specificity to HCs and speed of IR spectroscopy. Particular HC compounds are not analysed by fluorescence spectroscopy, but since the target compounds were intended to be found by GC-MS (see Ch. 3), such detail was unnecessary. Fluorescence spectroscopy conditions in the current study are given in Ch. 4, Table 4.1.

Appendix II:Examples of the Mass Spectra of Hydrocarbons

Fig. A2 shows some the mass spectra of three compounds, prominent in petrol and Outboard Motor Exhaust (OME): (a) benzene; (b) 1,3,5-trimethyl-benzene; (c) hexadecane. These compounds were also used as standards, for the calibration of the GC-MS apparatus used in this study. Each of the three diagrams includes actual mass spectra, as detected by the mass spectrometer (top), matching library mass spectra (centre) and molecular structures (bottom). In both the actual and library mass spectra, the mass/charge (m/z) ratio of the most prominent ionic fragments is shown.

In every mass spectrum, characteristic ionic fragments predominate, and in the case of benzene (Fig. A2(a)), most of the molecules only fragment to a very limited extent (see Reaction A1), as the benzene ring, found in all aromatic compounds, is a stable chemical structure.

Reaction A1

C_6H_6	→	$C_6H_6^+$	+	e^-
benzene	(ionised)	benzene molecular ion	+	electron

Most of the molecules are detected as the benzene ($C_6H_6^+$) molecular ion at m/z 78 and this gives rise to the corresponding large peak. Another prominent peak is situated at m/z 77, and is the phenyl ion. This is produced by reaction A2.

Reaction A2

C_6H_6	→	$C_6H_5^+$	+	H°
benzene	(ionised)	phenyl ion	+	Hydrogen radical

A few other ionic fragments are also detected, in much smaller quantities.

1,3,5-trimethyl-benzene is an alkylated aromatic compound, also containing a benzene ring which is very stable. However, the methyl groups present allow the molecule to shatter by means of several different pathways. The most prominent ionic fragments in the mass spectra of all alkylated benzenes and in benzene itself form a linear series which can be expressed as the formula $C_6H_5(CH_2)_n^+$. In the case of trimethylbenzene, a series of four such peaks occurs: those of m/z 77, 91, 105, and 119. These peaks correspond to n values of 0, 1, 2 and 3 in the above formula. Reactions A3-A6 show how these four characteristic ionic fragments are formed.

Reaction A3

C_9H_{12}	→	$C_9H_{11}^+$	+	H°
trimethylbenzene	(ionised)	dimethyl tropylium ion	+	Hydrogen radical

Reaction A4

C_9H_{12}	→	$C_8H_9^+$	+	$CH_3°$
trimethylbenzene	(ionised)	methyl tropylium ion	+	methyl radical

Reaction A5

C_9H_{12}	→	$C_7H_7^+$	+	$H° + C_2H_4$
trimethylbenzene	(ionised)	tropylium ion	+	Hydrogen radical + ethene

Reaction A6

C_9H_{12}	→	$C_6H_5^+$	+	$3CH_3°$
trimethylbenzene	(ionised)	phenyl ion	+	3 methyl radicals

The tropylium ion and its derivatives are unusual but relatively stable structures, being 7C aromatic rings, instead of a conventional 6C benzene ring with a CH_2 group attatched to it, which is more unstable. Hence the $C_7H_7^+$ cation is more likely to exist in the form of the tropylium ion (McLafferty, 1980). The molecular ion of trimethylbenzene is also prominent at the m/z value of 120.

Hexadecane is an aliphatic compound and its molecules behave very differently from those of aromatics, when it is shattered in the mass spectrometer. Larger aliphatic compounds have many isomers (molecules with the same chemical formula but different molecular structure) and the compound in this case is n-hexadecane or "normal" hexadecane, meaning that it does not have any side chains. The molecule has a roughly uniform stability along its length and it can fracture in many places. Fig. A2(c) shows a mass spectrum of hexadecane and a series of peaks at regular m/z intervals of 14 are evident, with the largest peaks at m/z 57, 71, 85 and 99. These peaks correspond to fragments of butyl ($C_4H_9^+$), pentyl ($C_5H_{11}^+$), hexyl ($C_6H_{13}^+$) and heptyl ($C_7H_{15}^+$) ions which are found in large quantities. Smaller amounts of other fragments are detected and a small proportion of the hexadecane molecules fragment into the molecular ion of hexadecane plus an electron, giving a small but obvious peak at m/z 226. The molecular ion peak is important in the identification of all compounds, both aromatic and aliphatic.

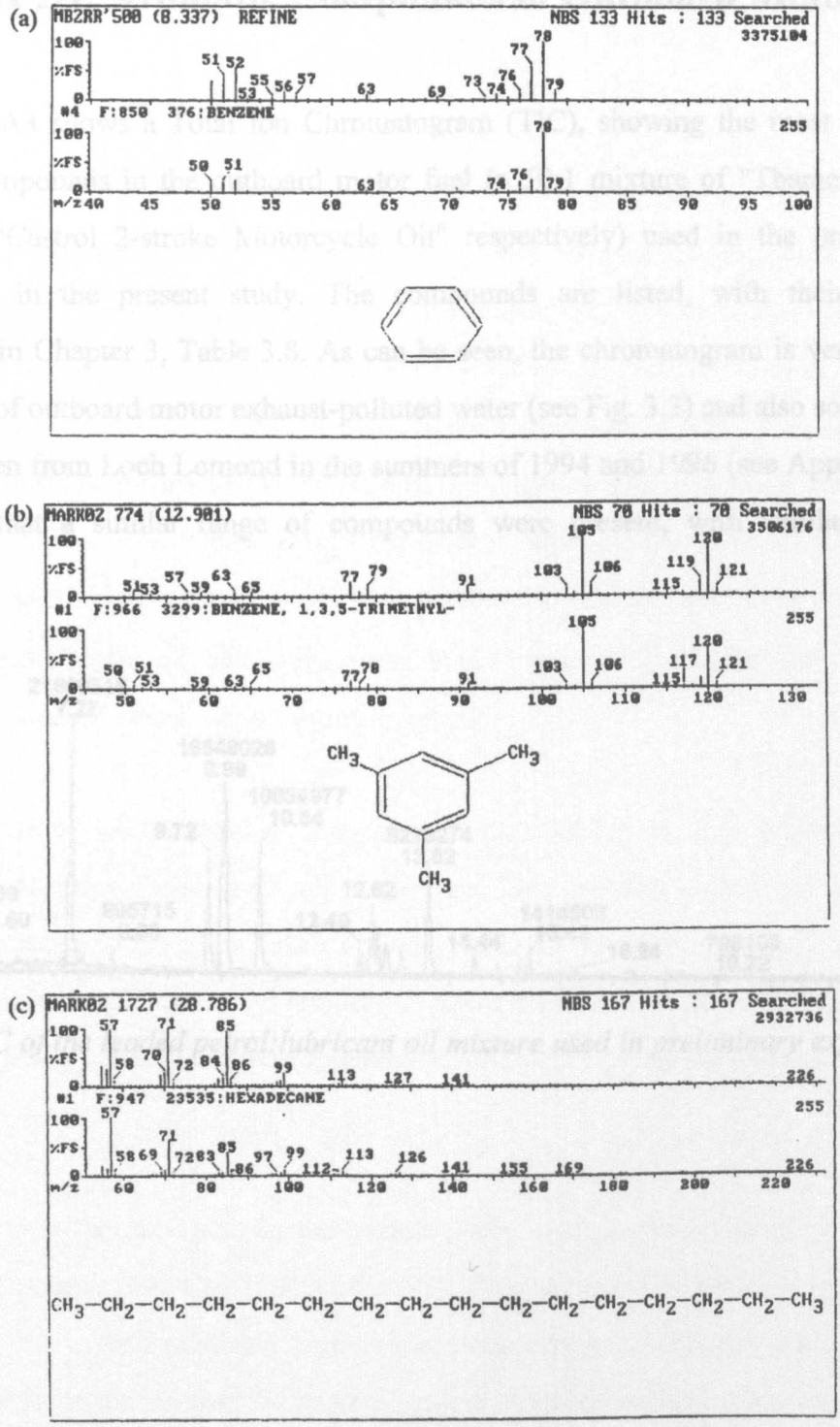

Fig. A.2: *Mass Spectra of three compounds: (a) benzene; (b) 1,3,5-trimethyl-benzene; (c) n-hexadecane. For each molecule, the actual mass spectra, library mass spectra and molecular structure are shown.*

Appendix III: Aromatic Compounds in Outboard Motor Fuel

Fig. A3 shows a Total Ion Chromatogram (TIC), showing the most abundant aromatic compounds in the outboard motor fuel (a 50:1 mixture of "Thames" leaded petrol and "Castrol 2-stroke Motorcycle Oil" respectively) used in the preliminary experiments in the present study. The compounds are listed, with their relative proportions in Chapter 3, Table 3.8. As can be seen, the chromatogram is very similar both to that of outboard motor exhaust-polluted water (see Fig. 3.3) and also some of the samples taken from Loch Lomond in the summers of 1994 and 1996 (see Appendix V), suggesting that a similar range of compounds were present, with similar relative proportions.

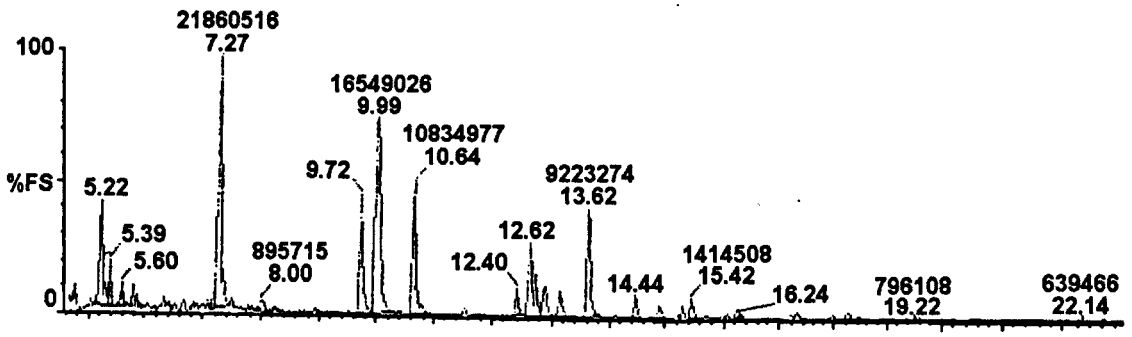

Fig. A3: TIC of the leaded petrol:lubricant oil mixture used in preliminary experiments in this study.

Appendix IV: Aliphatic Compounds in Exhaust-Polluted Water

Fig. A4 shows a Partial Ion Chromatogram (PIC) using only the ion fragment of m/z 57 which is specific for the detection of aliphatic compounds (particularly heavier compounds, with 10 carbon atoms or more). PICs have very low detection limits, much lower than those of TICs. This particular PIC shows that aliphatics are clearly present, but only in trace quantities. Table A4 lists the most abundant aliphatic hydrocarbons in EPW. Most of these form a linear series from the retention times of 14.15 min to 37.56 min, corresponding to the normal alkanes (n-alkanes: straight chain alkanes with no side branches) from C_9H_{20} (nonane) to $C_{20}H_{42}$ (icosane), respectively. This linear series may indicate the lighter aliphatic compounds in the lubricant oil and was not obvious in the PIC from the unburned fuel:oil mixture sample.

A "hump" of unresolved complex mixture (UCM) is also present, located between the retention times of 45 min to 60 min. This probably represents the heavier aliphatic compounds found in lubricant oils, with 25-40 Carbon atoms. The UCM results from the large number of isomers of these heavier compounds, which form so many small peaks at many different retention times that the peaks become indistinct and overlap.

Although the apparatus in the current study was not ideal for the analysis of aliphatic compounds (See Ch. 4, Section 4.4.3), they were clearly detected in the EPW. This may be due to their relatively high overall concentrations, although it also could be evidence that lubricant oil may be relatively more abundant after combustion. Previous research (Bartlett, 1989) has shown that lubricant oil consists largely of aliphatic HCs with 9 to 40 Carbon atoms.

Table A4: Identifiable aliphatic compounds present in EPW.

RT	Compound	Chemical formula
14.15	n-nonane	C_9H_{20}
21.47	n-dodecane	$C_{12}H_{26}$
23.89	n-tridecane	$C_{13}H_{28}$
26.16	n-tetradecane	$C_{14}H_{30}$
28.32	n-pentadecane	$C_{15}H_{32}$
30.36	n-hexadecane	$C_{16}H_{34}$
32.29	n-heptadecane	$C_{17}H_{36}$
34.12	n-octadecane	$C_{18}H_{38}$
37.56	n-icosane	$C_{20}H_{42}$

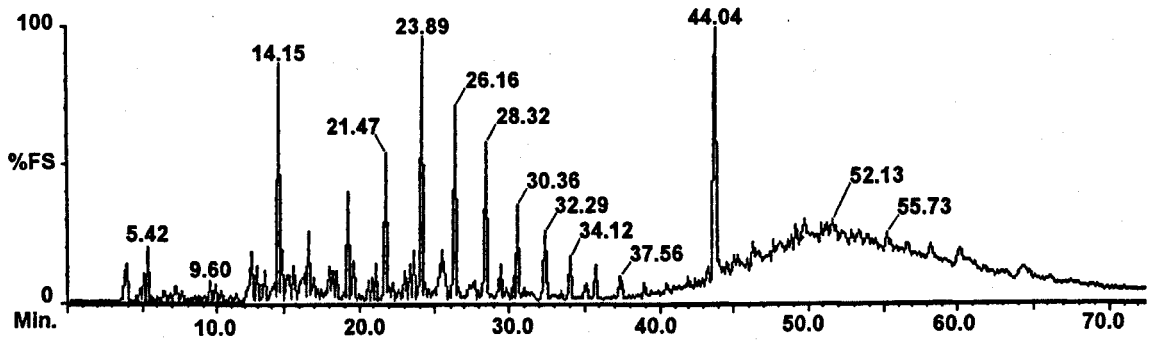

Fig. A4: Partial Ion Chromatogram (PIC) of ion (m/z) 57, of EPW, produced in preliminary experiments in the current study.

Appendix V: Organic Compounds in Water Samples from Loch Lomond

A5.1: Preliminary 1994 Survey

Fig. A5.1 shows complete Total Ion Chromatograms (TIC's), i.e. up to Retention Times (RT's) of 70 min., of the surface water samples from two sites in Loch Lomond (in the preliminary experiments, using the conditions in Table 3.1) taken on Sunday August 7, 1994: Balmaha Pier and the North Narrows. Samples were taken just after peak powerboating activity on an appropriate weekend day in summer, with high boat density on Loch Lomond on that day. Table A5.1 shows all of the compounds detected at these two sites with RT's.

Fig. A5.2 displays part of the chromatogram of the reagent blank taken at the time of analysis in August 1994 and shows low levels of some contaminants including methyl-benzene (toluene).

The reagent blank also contains traces of a compound, likely to be a phthalate (see Fig. A5.3 for the relevant section of the chromatogram). Phthalates are found in plastics and are nowadays considered to be ubiquitous environmental contaminants (Best and Dawson, 1993). However, comparatively high (peak height over 70 times higher) levels of the same compound (similar retention time of approximately 47.0) were found in two of the samples taken from Loch Lomond, indicating that it was present in the water. Another similar compound, possibly a detergent, absent in the reagent blank was also found at in several water samples. The two compounds may have emanated from boat cleaning operations.

Table A5.1: Compounds shown in the complete TIC's of surface water in Balmaha Pier and the North Narrows on Sunday August 7, 1994. Compounds with confirmed identity are underlined.

Compound	RT (Balmaha)	RT (Narrows)
Solvent	4.90	4.95
Methyl Benzene (toluene)	9.12	9.12
7C alkene	10.55	
1,4 Dimethylbenzene		12.02
1,2,4 trimethylbenzene		15.82
11C alkane	18.40	
Naphthalene	21.57	21.60
Detergent compound	30.79	30.80
Phthalate compound	47.04	47.01

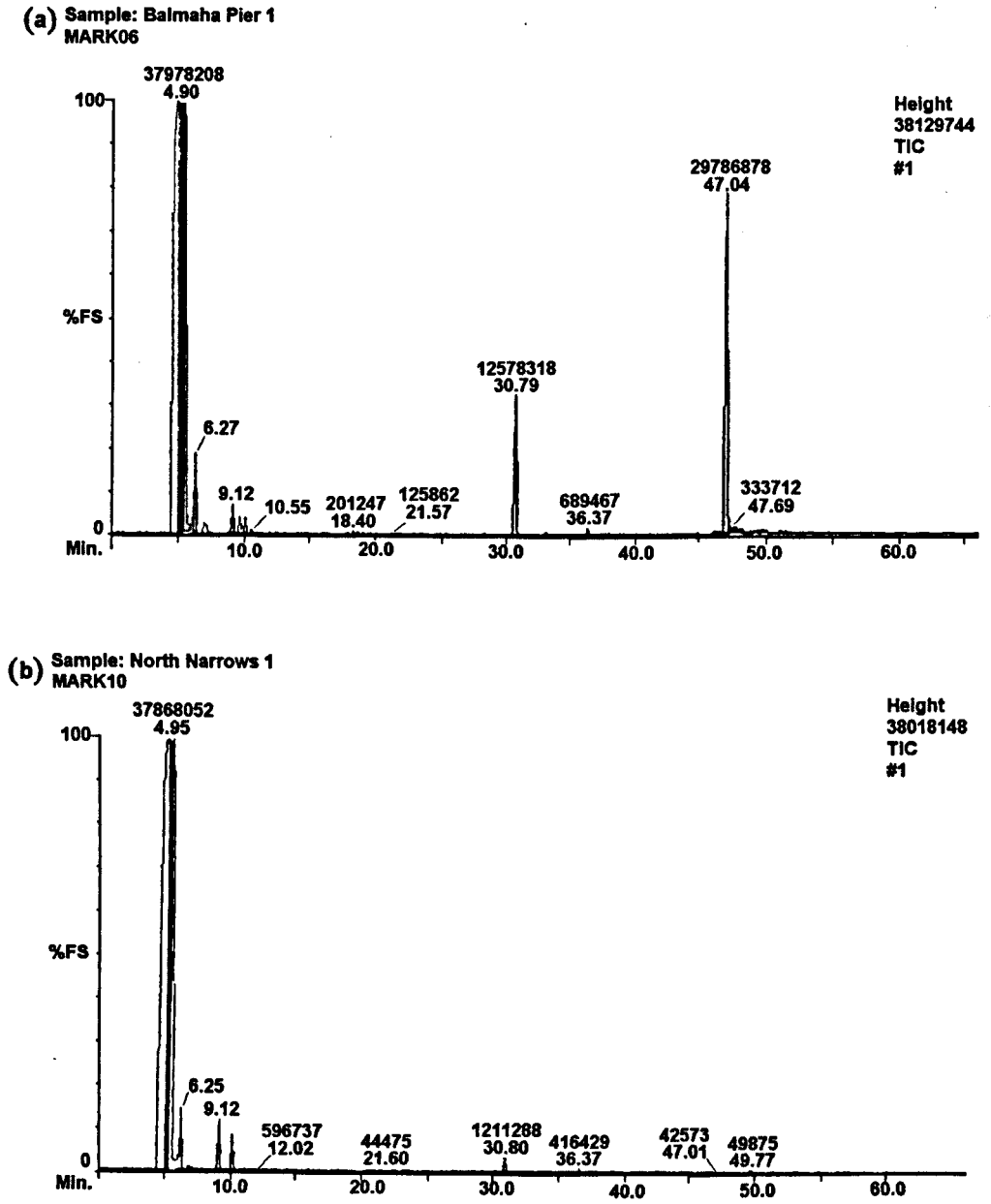

Fig. A5.1: Entire chromatograms (up to RT 70 min.) of organic chemicals found on Sunday August 7 1994, in the surface waters of (a) Balmaha Pier; (b) The North Narrows.

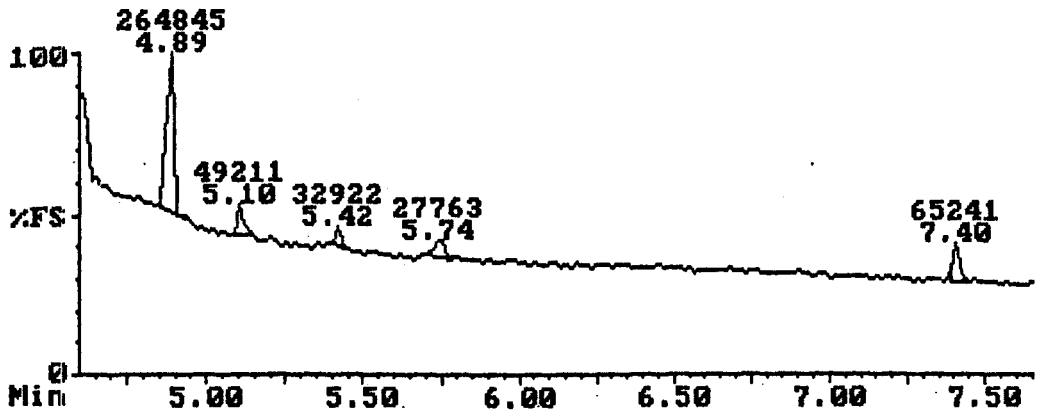

Fig. A5.2: Section of the TIC of the blank, run at the same time as the August 1994 samples, showing traces of toluene present (peak with RT of 7.40 min)

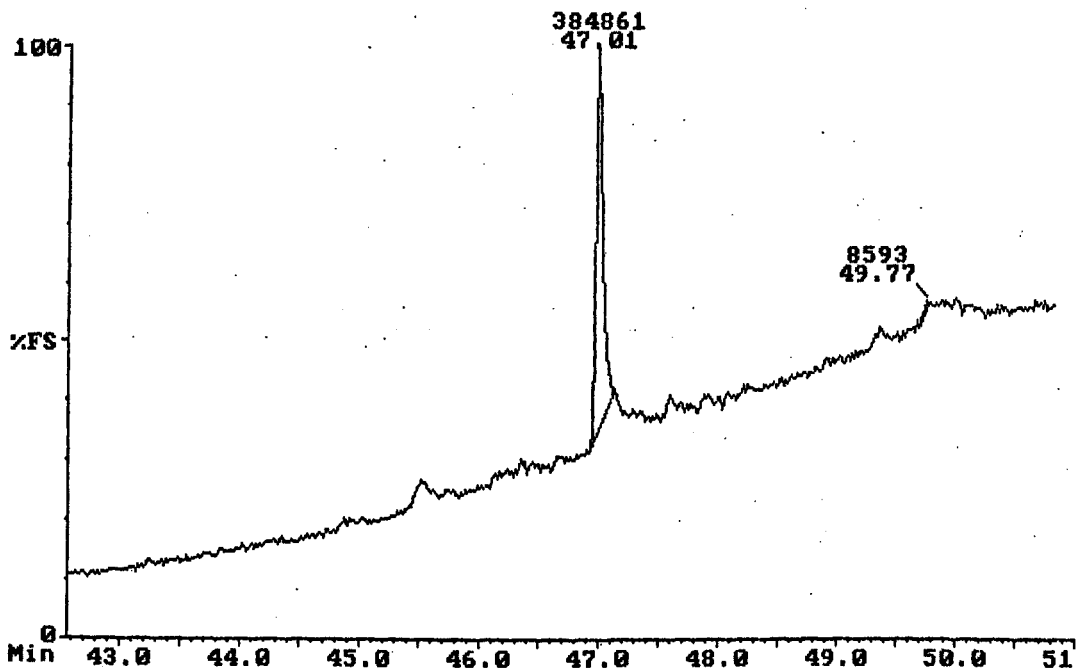

Fig. A5.3: Section of the TIC of the blank, run at the same time as the August 1994 samples, showing a phthalate compound present (peak with RT of 47.01 min)

Fig. A5.4 shows a magnified portion of the chromatogram of Balmaha Pier surface water (shown in full in Fig. A5.1). Six volatile aromatic hydrocarbon compounds can be seen and these are listed (with RT's corresponding to those indicated in Fig. A5.4) and identified in Table A5.2. There are some chlorinated compounds in Table A5.2, which may have been impurities in the dichloromethane itself. These compounds were also found in the solvent blanks, analysed in this study.

Table A5.2: Compounds shown in the magnified portion of the chromatogram of surface water from Balmaha Pier. Peak heights are in ion counts, used in the computer quantification programme. Chlorinated compounds are contaminants found in low concentrations within the solvent itself. Compounds with confirmed identity are underlined.

Compound	RT	Peak Height (thousands)
Chlorinated compound	6.27	7007
Benzene	6.83	910
Chlorinated compound	6.92	909
5C unsaturated alcohol	7.22	700
Methyl benzene (toluene)	9.12	2689
7C alkene	9.73	1415
Chlorinated	10.17	1300
7C alkene	10.55	433
Ethyl benzene	11.75	97
1,4 dimethyl benzene	12.02	322
1,2 dimethyl benzene	12.72	81
10C cyclopropane	13.42	147
Chlorinated compound	13.50	81
10C cyclohexane	14.35	121
1 ethyl- 3 methyl benzene	14.79	57

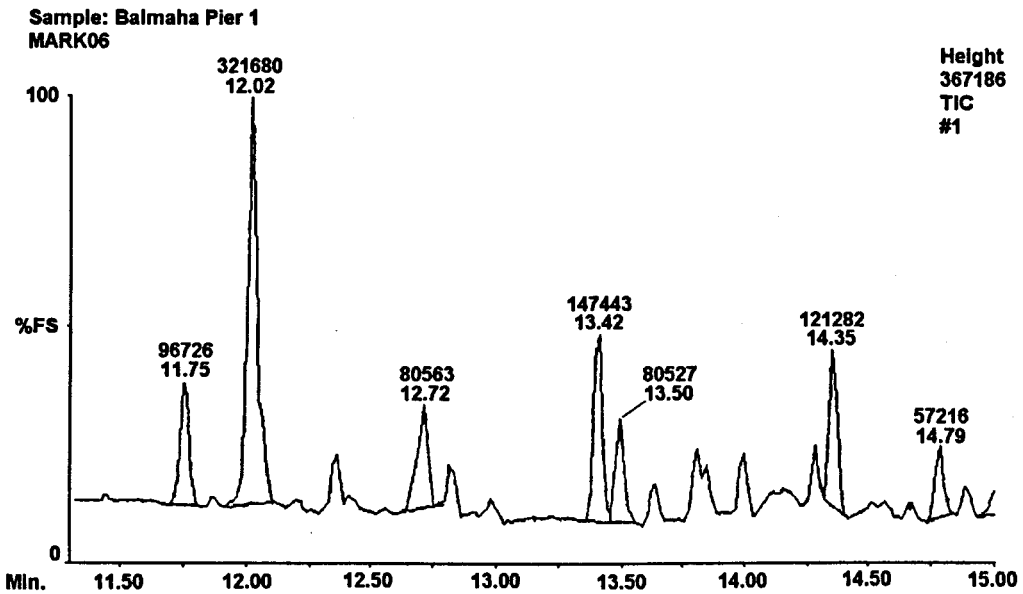

Fig. A5.4: Magnified portion of the chromatogram for the hydrocarbons in the surface waters of Balmaha Pier, from RT 11 min. to 15 min.

A5.2: 1996 Survey

Volatile aromatic hydrocarbons (HCs) were also detected in water samples (both surface and sub-surface) taken from Loch Lomond during the summer of 1996. The sites are numbered as in Table 5.1 and Fig. 5.2. The analytical conditions are described in Chapter 4, Section 4.3.8 and Fig. A5.5 and A5.4 show chromatograms of the extracts of two of these samples:

Fig. A5.5: Leven Entrance (Site No. 9), surface water, sampled on Sunday July 21, 1996

Fig. A5.6: Balmaha Pier (Site No. 6), surface water, sampled on Saturday August 3, 1996

Sections (showing some but not all compounds present) of both PICs (for ionic fragment 105, which is diagnostic of C8 and C9 aromatic HCs) and TICs are presented for both samples and GC-MS analytical conditions are shown in Chapter 4, Table 4.3. Table A5.3 lists the compounds present, and where known, their aqueous concentrations. The total concentration of volatile aromatic HCs, determined by

fluorescence spectroscopy, is also given for each sample. The retention times correspond to those given in the chromatograms.

As in 1994, toluene was detected in the blank. However, the measured concentration was much higher (1.15 $mg.l^{-1}$) than the estimated concentration in the 1994 blanks (almost certainly less than 0.2 $mg.l^{-1}$). In addition the concentration factor of samples during the extraction process was only 180 in 1996, compared to 900 in 1994. This means that the detection limits for toluene in 1996 were far higher than in 1994 and correspond to relatively high levels in environmental samples (approximately as high as the actual concentrations measured in 1994). Analysis of toluene was therefore not feasible in any of the eight samples taken in 1996.

Both samples contain volatile aromatic HCs, indicating contamination by petroleum hydrocarbons, either from petrol, diesel or lubricating oil. The chromatogram of the sample from the Leven in particular contains the same compounds with very similar relative proportions to the volatile aromatics of petrol (see Johansen *et al.*, 1983). This strongly suggests that the source of contamination in this sample is almost exclusively petrol. By contrast, the Balmaha sample contains some different aromatic compounds and different proportions of those compounds common to both samples. Further research is necessary, to ascertain the sources of these compounds, although they are likely to be boat-derived.

Table A5.3: Compounds (shown in the chromatograms) detected in surface water from the Leven Entrance (sampled on July 21, 1996) and Balmaha Pier (sampled on August 3, 1996). The retention times (in this case identical for both samples) correspond to those indicated on the chromatograms in Fig. A5.5 and A5.6 respectively. Aqueous concentrations of compounds (where known) and the total quantity of volatile (monocyclic) aromatic HCs (measured by fluorescence spectroscopy) are also given.

Compound	RT	Quantity at Leven on 21.7.96 ($\mu g.l.^{-1}$)	Quantity at the Balmaha on 3.8.96 ($\mu g.l.^{-1}$)
Ethyl Benzene	10.45		
1,3- and 1,4- dimethyl-benzene	10.65	1.27 (1,3-dimethyl-benzene)	0.49 (1,3-dimethyl-benzene)
1,2-dimethyl-benzene	11.31	0.45	1.36
Propyl-benzene	12.96		
1-ethyl- 3-methyl- benzene	13.17		
1,3,5-trimethyl-benzene	13.36		0.24
1-ethyl- 2-methyl-benzene	13.69		
1,2,4-trimethyl-benzene	14.06		
1,2,3-trimethyl-benzene	14.86		
Total concentration of volatile aromatic HCs.	-	3.32	10.60

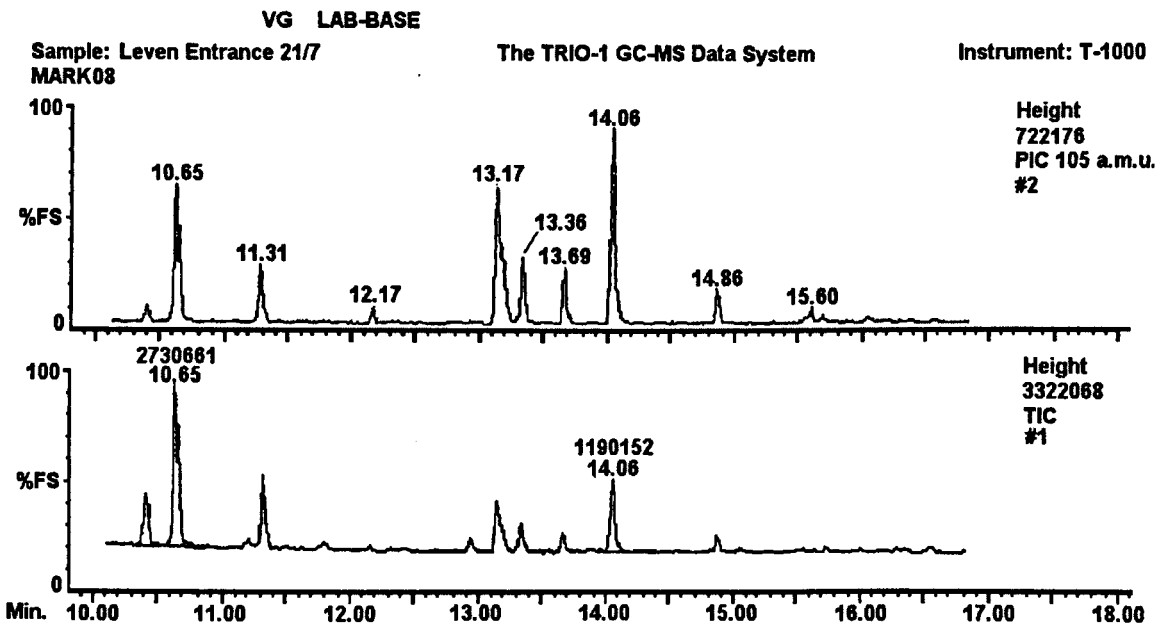

Fig. A5.5: TIC and PIC of the sample extracts of surface waters of the Leven Entrance, sampled on 21st of July, 1996. Table A5.3 lists the compounds detected.

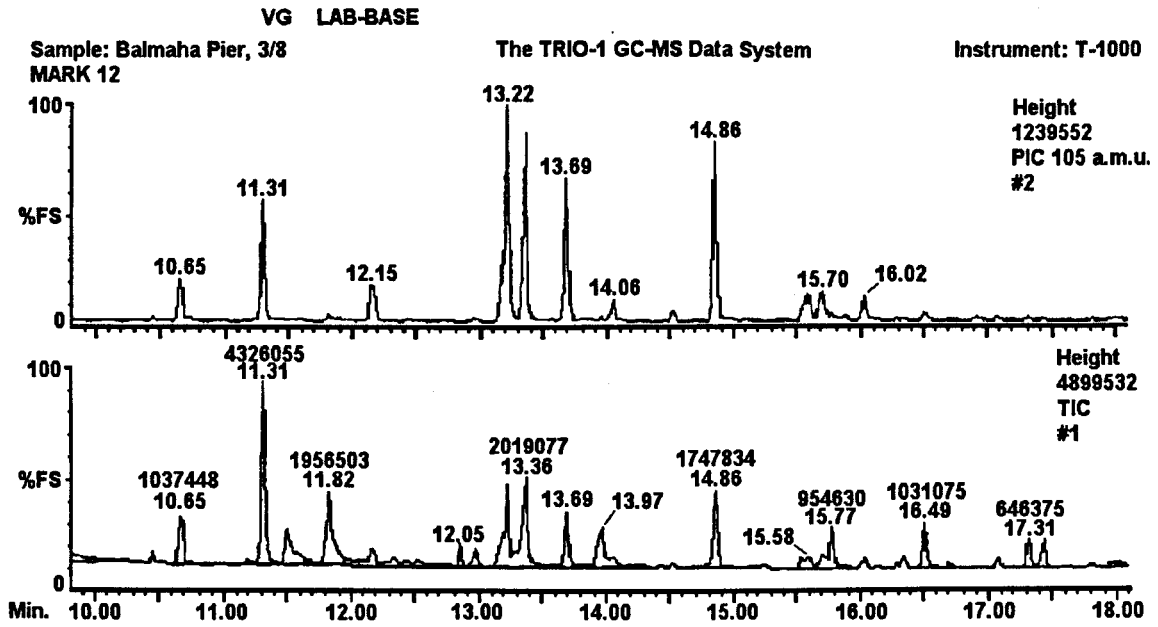

Fig. A5.6: TIC and PIC of the sample extracts surface waters of Balmaha Pier, sampled on 3rd of August, 1996. Table A5.3 lists the compounds detected.

Appendix VI: Evidence for the Effectiveness of the Sample Purification Procedure in the Current Study (Additional to that Presented in Chapter 4)

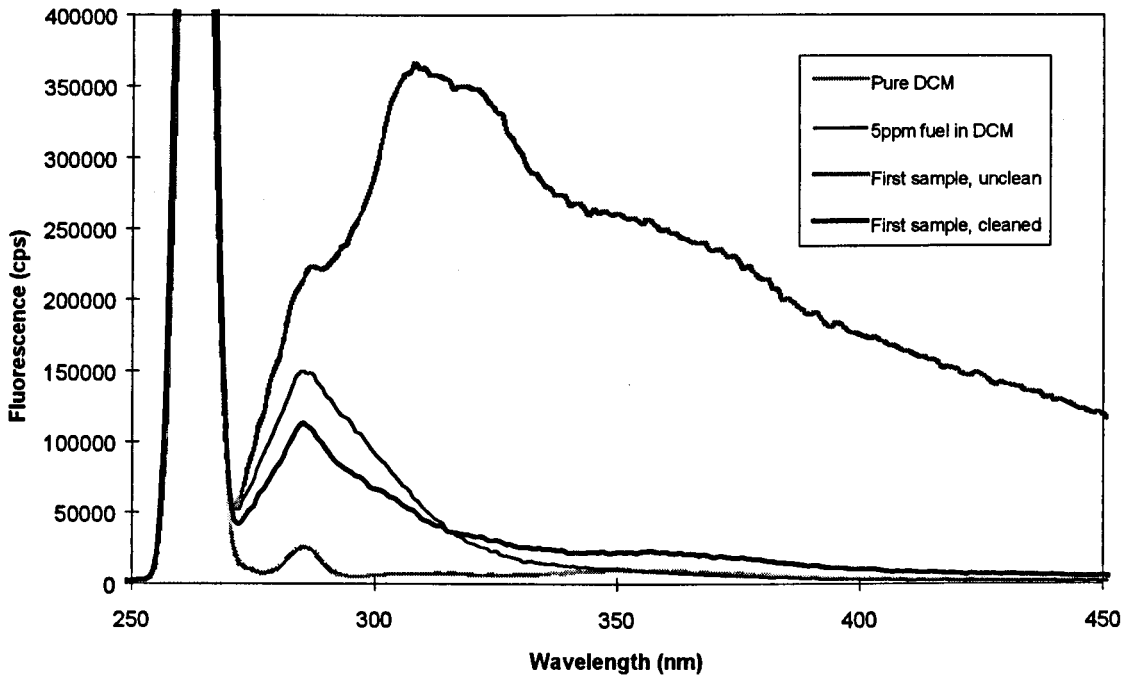

Fig. A6.1: Effectiveness of sample clean-up, showing scans of pure DCM, 5 mg.l-1 fuel:oil mixture in DCM and one sample from Loch Lomond, before and after clean-up.

Fig. A6.1 and Fig. A6.2, provide evidence for the effectiveness of the clean-up procedure in facilitating the measurement of low concentrations of hydrocarbons. In Fig. A6.1, both cleaned and uncleaned fluorescence emission scans (excitation wavelength 262 nm) of a sample taken from a site in Loch Lomond during the summer of 1996 are compared to scans of pure DCM and of a standard solution of fuel:oil mixture at a concentration of 5 $mg.l^{-1}$ in pure DCM (approximately 1.63 $mg.l^{-1}$ of volatile aromatic HCs). In the case of the uncleaned sample, a large amount of background fluorescence is evident at almost all wavelengths scanned, preventing any meaningful analysis. However the cleaned sample shows a scan very similar to that of pure fuel:oil mixture in DCM, indicating some contamination by aromatic hydrocarbons.

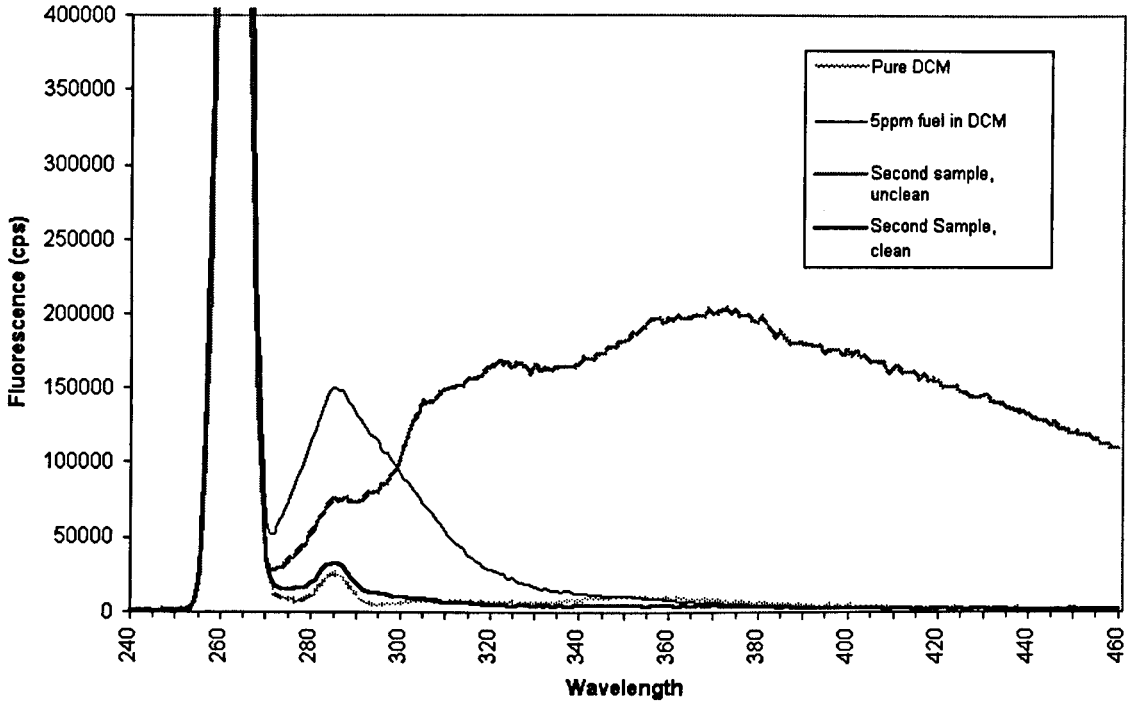

Fig. A6.2: Effectiveness of sample clean up, showing scans of pure DCM, 5 mg.l-1 of fuel:oil mixture in pure DCM and of a second sample from Loch Lomond, before and after clean-up.

Fig. A6.2 shows emission scans of a second sample, taken from Loch Lomond during the same summer, both before and after clean-up and also compares these scans with those of pure DCM and the 5 $mg.l.^{-1}$ standard of fuel:oil mixture (1.63 $mg.l.^{-1}$ of volatile aromatic HCs) in pure DCM. The cleaned sample from the loch in this case produces a scan very similar to that of the sample of pure DCM with a scarcely elevated fluorescence reading at 286 nm of 32200 cps. This reading corresponds to an almost undetectable concentration of volatile aromatic HCs. For comparison, the detection limits of the method are 29697 cps (as described in Chapter 4, Section 4.3.7(a).2), corresponding to an aqueous concentration of volatile aromatic HCs of 0.4 $\mu g.l.^{-1}$. The evidence presented here suggests that negligible levels of volatile aromatic HCs were found in this second sample.

Appendix VII: Evidence for Diesel Pollution in Loch Lomond

The fluorescence technique developed in the present study (Ch. 4) was used to detect and quantify volatile aromatic HCs from outboard motor fuel. These compounds are monocyclic (having only one benzene ring), being benzene (C_6H_6) and its derivatives (alkylated benzenes) and comprise 25 – 45 % of petrol by weight (Johansen *et al.*, 1983), which in turn comprised 98 % (v/v) of the 50:1 fuel:lubricant oil mix used to propel the outboard motor used in experiments in the present study.

Almost any petroleum oil, including crude oil, lubricant oils and diesel oil, can be analysed by fluorescence spectroscopy, because all such oils contain aromatic HCs, either monocyclic or polycyclic. The latter consist of more than one benzene ring joined together and are known as PAHs (polycyclic aromatic hydrocarbons). The simplest PAH compound is naphthalene ($C_{10}H_8$), consisting of two benzene rings. Previous studies (MEWAM, 1988) have given details of methods used for the analysis of petroleum oils other than petrol (gasoline).

In the present study, a number of water samples taken from Loch Lomond during the summer of 1996 contained detectable levels of volatile aromatic HCs. In nearly all cases, the primary source of these compounds was either petrol spillage or exhaust emissions from petrol powered boats (either two-stroke or four-stroke outboard motors, or inboard motors). However, four of the water samples contained higher levels of 30 $\mu g.l^{-1}$ or more and three of these latter samples manifested markedly different fluorescence scan patterns from petrol or outboard motor exhaust-polluted water (EPW). All three of these latter samples were of surface water taken from areas where oily slicks were present (which happened on two occasions). On one of these occasions, a large tour boat was idling nearby and appeared to be discharging unburned fuel from its engine (which was probably diesel-powered).

In order to investigate the possibility of the oily slicks being diesel, a sample of "Thames" diesel was obtained from the Glasgow University Garage. Standards of pure diesel in pure dichloromethane (DCM, the extraction solvent used in this study) were

made up, with concentrations ranging from 0 to 100 $mg.l^{-1}$ in DCM. The samples and standards were analysed by fluorescence spectroscopy with an excitation wavelength of 262 nm, and also by synchronous scanning fluorescence spectroscopy (SSFS) with an offset of 50 nm. Prior to analysis, the samples and standards were purified by normal-phase column chromatography, to remove lipids, amino acids and humic acids. These compounds are present in water and can cause interference in the fluorescence analysis of HCs if they are not removed. The conditions used were appropriate for detection of petrol and not modified in any way for diesel. More details are given in Ch. 4 (see Tables 4.1 and 4.3).

Fig. A7 depicts the fluorescence emission spectra (excitation wavelength 262 nm) of 100 $mg.l^{-1}$ pure diesel in pure DCM and also that of one of the three water samples taken from the locations with slicks present. The sample here was surface water, taken at the Narrows on Tuesday 13th of August 1996 at 5.00 p.m. As can be seen, there is an almost perfect match between the scans. The same situation pertained for the other two samples and also for all three samples, when analysed by SSFS. This shows that the two slicks observed in Loch Lomond in the summer of 1996 were almost certainly diesel fuel. The emitted fluorescence is found at greater wavelengths than that from monocyclic aromatic HCs, and may emanate from PAHs. Previous research has shown that diesel contains PAHs (NRC, 1985) and that these compounds emit fluorescence at these higher wavelengths (Siron *et al.*, 1991; López de Alda-Villaizan *et al.*, 1995). Unfortunately, it was not possible to fully confirm these findings, using GC-MS analysis.

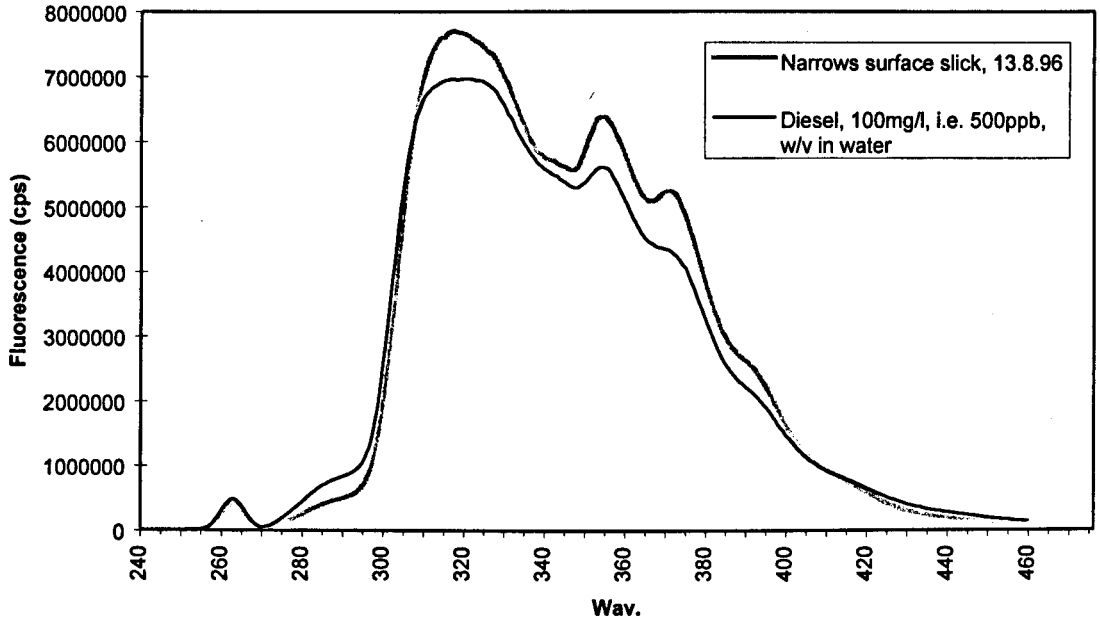

Fig. A7: *Fluorescence emission scans of pure diesel (100 mg.l^{-1} in DCM) and an extract of a sample of surface water taken from Loch Lomond at the Narrows on 13.8.96 at 5.00 p.m. An oil slick was present there at that time (NB: the peak at 262 nm is caused by some of the excitation light reaching the emission detector).*

Presuming that diesel was present, it is possible to gain an approximate indication of the concentration of diesel-derived HCs in the surface water samples. The standard of 100 mg.l^{-1} in pure DCM shows similar levels of fluorescence (indicated by similar peak heights) as all of the scans. This indicates that the levels of aromatic HCs are approximately similar in both samples, being about 38.2 mg.l^{-1} in DCM (diesel is about 38.2 % aromatic – see NRC, 1985). If it is assumed that all of the aromatic HCs in the water samples were extracted (this gives a conservative estimate of aqueous HC concentrations), 38.2 mg.l^{-1} equates to an aqueous aromatic HC concentration (in the surface water samples from Loch Lomond) of 191 μg.l^{-1}. These conclusions are tentative, and further work would be useful to properly investigate diesel pollution in Loch Lomond, particularly its vertical distribution.